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THE EFFECTS OF RADIATION ON THE DEVELOPMENT OF *TRICHINELLA SPIRALIS*

WITH RESPECT TO ITS APPLICATION TO THE TREATMENT OF OTHER PARASITIC DISEASES

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Since radium has been shown by biological experiment to have a pronounced effect on the development of the germ cells of various species, the possibility of its utilization in the destruction or even in the emasculation of certain parasites for which there is at present no efficient remedy appears worthy of consideration. It was thought that radium might be employed to advantage in the treatment of cases of schistosomiasis in which the bladder is involved, several of which have been under the authors' observation for a considerable period of time. Although this condition is of common occurrence in certain parts of the world and although it is frequently attended with serious complications, up to the present time no successful form of treatment has been discovered. Since the inflammation in this disease is produced by the presence of the ova in the tissues and since the worms from which the latter are derived, are situated in close proximity to the mucous surface of the bladder, this mode of attack seemed to be especially appropriate. It did not appear justifiable, however, to undertake the treatment of human cases without a certain amount of preliminary experimentation.

While the use of Roentgen ray for the treatment of schistosomiasis has been suggested,[1] there appears to be an advantage in the use of radium or its emanation in this disease, for the bladder wall in which the worms are situated may be radiated directly from its inner surface and rays of shorter wave length may be utilized than is possible with the Roentgen rays. According to Packard (quoted by Abbe, 1914) the beta rays are more effective than the gamma rays in retarding the development of certain species.

1. The advisability of employing the x-ray therapeutically in this disease was discussed by Doctor R. Gonzales Rincones of Venezuela at the recent Pan-American Scientific Congress at Washington.

In the following experiments radium emanation was employed as follows:

1. To radiate from the outside the abdomen of rats previously fed with the cysts of *Trichinella spiralis*.

2. To radiate muscle containing encysted larvae of this parasite.

3. To radiate the worms directly during their development in the intestine by feeding minute glass tubes containing radium emanation.[2] Radiation was accomplished in several ways and further details will be presented with the account of each experiment.

Technic.—The effects of radiation on the parasite were judged by either the failure of the larvae to develop in the intestine of rats and mice or by abnormalities in their development. It was thus important to determine the number of worms present in the intestine and also to note any retardation in their differentiation or growth. In order to count the worms, the intestine of the animal to which the larvae had been fed was cut into pieces of from 3 to 4 cm. in length. These were each placed on an ordinary microscopic slide, opened with fine scissors, and by fixing one end of the piece with tweezers the mucosa was completely stripped from the muscular wall by several light sweeping strokes with the edge of a scalpel. The material obtained, i. e., mucosa and softer portions of the intestinal contents, was spread slightly, and then pressed gently beneath a large 22 by 40 mm. cover glass. With a microscope equipped with a mechanical stage, all the worms in such preparations may be readily observed and counted. Since the material is flattened into a thin film the anatomy of the worms is clearly apparent so that an accurate enumeration of the sexes may readily be made. In the earlier experiments equal amounts of muscle taken from corresponding portions of the body of an infected animal were used for infecting the radiated and the control series, respectively. Since this procedure furnished only approximately equal dosage in the later experiments with mice, the cysts contained in strips of diaphragm were counted and an equal number fed to each of a series of animals.

Observations made during the course of this study failed to confirm certain statements, which occur quite generally in standard works, concerning the anatomical distribution of *Trichinella spiralis*, the ratio of the sexes, and the span of life of the adult male and female of this species.

Distribution in Rats and Mice.—Trichinellae are said to mature in the duodenum and jejunum and it might be inferred that the adults are confined to the first portion of the intestine. In the course of the

2. The authors are indebted to Doctor William Duane for collecting and measuring the radium emanation used and also for suggestions as to dosage, filtration, etc.

following experiments the worms were comparatively rarely present in the first portion of the small intestine of rats and mice, but were found in great numbers throughout the remainder. They not infrequently occur also in the cecum and colon of mice and occasionally in the large intestine of rats. It appears probable that the small size of these host species may account for the presence of the worms in the large intestine since no great extent of gut would have to be traversed before reaching the cecum.

Sex Ratio.—According to Stäubli (1909), great discrepancies with respect to this point are found in the statements of different authors. Thus Leuckart reports the females as greatly in excess of the males, in one instance in a 10:1 or 20:1, and in another instance in a 6:1 ratio. Zenker calls attention to the difficulty in finding the males on account of their smaller size. Askanazy, on the other hand, finds the males greatly in excess in the intestinal contents, but this was thought to be due to the fact that the females burrow into the mucosa, while the males remain free. Ostertag claims that the males and females are originally present in equal numbers, but that the former after copulation diminish in number, so that after 10 to 14 days only females are present. Both sexes were observed by Pagenstecher 56 days after ingestion. Stäubli notes great variation in the sex ratio in different cases with respect not only to the mature adults, but also to the encysted larvae the sex of which he is able to distinguish. He is unable to account for this lack of uniformity in the relative number of the sexes.

In order to avoid error in estimating the relative number of males and females it is important to examine the material in such a way that none will be overlooked. The males, on account of their smaller size, are not so readily detected, except with the aid of a microscope. Thus in 100 worms picked out with the naked eye from a suspension of intestinal contents and mucosa, not a single male was found; whereas a count made with the microscope of samples of the same material showed 31 per cent males

Counts of 100 or more worms from the intestines of four rats of the present series showed the percentage of males to vary from 31 to 41 per cent seven or eight days after injestion. In a total of 446 worms, 160, or 36 per cent, were males. An approximation of a 1:2 ratio was thus found in these animals. Rats killed seventeen or eighteen days after ingestion of infected muscle showed practically the same ratio, although only few worms were found. It is of interest to note that in one rat in which a single male was found unaccompanied by any females, numerous larvae were found in the striated muscles showing that this male had outlived one or more females which had been present.

EXPLANATION OF PLATE

Fig. 1.—A cross section of male and female *Schistosoma haematobium* situated in a distended vein at the juncture of the submucosa and muscular wall of the bladder. This vessel is evidently occluded by the inflammation which the worms' presence has excited. The intestinal ceca of the female are distended with deeply stained material to the right of which is the ovary.

Fig. 2.—Male and female *S. haematobium* in longitudinal section. In the upper portion of the sectioned worms to the right a row of eggs is visible in the tubular uterus of the female. The acetabulum of the male is apparent, directed inwardly near the anterior extremity of the worms at the left. These worms are situated in veins beneath the submucosa, in this instance 4 or 5 mm. from the surface of the mucosa. The inflammatory changes in the latter are apparently due to the presence of numerous ova, for these are found surrounded by collections of exudate with which they are evidently discharged during the contraction of the bladder. Many ova also fail to reach the surface but become imbedded in the tissue where they are eventually destroyed, the shells persisting as foreign bodies.

PLATE

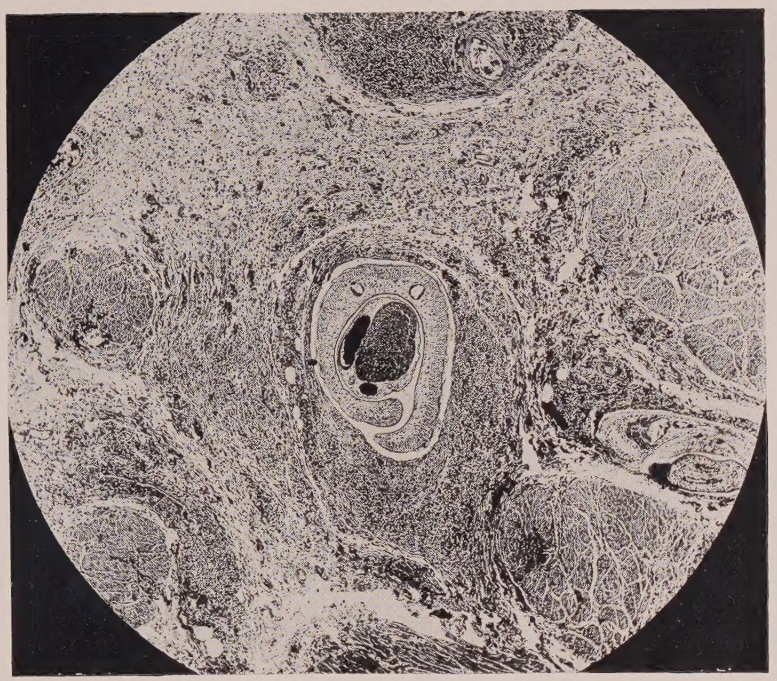

Figure 1

Figure 2

In mice fed with relatively small numbers of encysted larvae and killed four, five, six, seven and nine days later, there is considerable variation in the sex ratio, evidently on account of the small numbers of adult worms present in each animal. Including trichinellae subjected to radiation, together with those of the control mice, there were 516 counted, and 159, or 30.8 per cent of these were males. Considering the non-radiated separately, there were 167, of which 42, or 25 per cent, were males. There was no marked diminution in the number of males from the fourth to the seventh day, and the number counted on the ninth day is too small to be of significance. The sex ratio of one male to two females is thus also approximated for this parasite in the mouse.

Disappearance of Adult Worms from the Intestine.—Whereas the males are said to diminish in number after copulation, which is accomplished by the second or third day after ingestion, it is stated that the females may persist for five weeks or longer. Cohnheim claims to have observed trichinellae in great numbers up to the seventh week; Kratz found them seventy-seven days, and Leuckart twelve weeks after ingestion. In experimental animals the embryos are said to be liberated from adults for five to seven weeks after the ingestion of trichinous meat (Fantham, Stephens, and Theobald, 1916). It would appear from the findings in the rats of the following experiments that the adult worms disappear much earlier than the above observations by various authors would indicate. The data collected are presented in the following table.

TABLE OF RESULTS

Rat	Period of Infection	Number of Adult Trichinae	Males	Females	Remarks
Wild 4853A	19 days	None			Only a portion of intestine examined
Wild 4853B	19 days	None			Only a portion of intestine examined
White 4965 A	25 days	Present	Present	Present	
White 5590	7 days	Numerous	31.5%	68.5%	146 counted
White 5592	17 days	One		One	Entire intestine examined
White 5618	7 days	Estimated 600	36%	64%	100 counted
White 5619	8 days	Estimated 800	37%	63%	100 counted
White 5620	8 days	Estimated 1,000	41%	59%	100 counted
White 5622	18 days	None			Ten inches of small intestine examined
White 5623	18 days	One	One		Ten inches of small intestine examined
White 5624	18 days	Two		Two	Ten inches of small intestine examined
White 5625	18 days	None			Ten inches of small intestine examined
White 5626	18 days	None			Ten inches of small intestine examined

Numerous larvae were found in the skeletal muscles of all the negative rats showing that the adult worms had been present in the intestine of each. In one rat adult worms of both sexes were still present twenty-five days after ingestion, but in general their paucity or absence is notable in the animals killed later than the sixteenth day. In the last group, each animal of which was fed an equal amount of trichinous muscle, the most striking differences are shown with respect to the number of worms present in the rats killed seven and eight days and in those killed eighteen days later. This would indicate that this parasite's span of life in the intestine of the rat is rarely over three weeks, although there may occasionally be individuals persisting longer.

In the following experiments radium emanation was used. Since this substance is transformed at a known rate, the amount of radiant energy available is constantly diminishing (approximately one half in four days), so that the dosage is greatest at the beginning of the exposure.

1. RADIATION OF THE ABDOMEN FROM THE SURFACE OF THE BODY

In considering the effects of radiation on *Trichinella spiralis*, both the failure to develop as determined by the number present and the retardation of development as indicated by the absence of worm-shaped embryos in the females seven days after feeding were taken into account. Although various degrees of maturity were met with at this time, the presence or absence of worm-shaped embryos could be readily determined and served as a useful, although arbitrary, index. Small and evidently poorly developed males were also met with, but since they furnish no prominent feature by which their stage of development could be judged, they are not in this respect taken into consideration.

Experiment 1

February 18, 1916. Equal amounts of muscle containing encysted larvae were fed to four healthy rats and two of these served as controls, while the other two were radiated from the surface of the abdomen. Radium emanation enclosed in capillary glass tubes with 0.1 mm. of steel, 1 mm. of silver and a layer of adhesive plaster for filtration was used. This applicator was moved each day to a new area on the abdominal wall, from the entire extent of which the fur had been removed. One rat, 5591, which was radiated in this manner with a tube of 8.8 millicurie strength from the second day following ingestion of trichina, died six days later; that is, seven days after infection. An additional tube containing 6.4 mc. was added five days after feeding and two days before death. Another rat, 5593, was radiated in a similar manner with a very weak tube (3 mc.) from the sixth to the tenth day and with a 9.6 mc. tube from the tenth to the seventeenth day, when it was killed and the number and condition of intestinal trichinae determined.

TABLE OF RESULTS—EXPERIMENTS 1 AND 2

Trichina-Fed Rat	Treatment	Death	No. of Worms Found			Remarks
			Total	Males	Females	
Experiment 1						
5590	None (control)	Killed 7 days later	Numerous..	31.5%	68.5%	Females 2% without embryos
5591	Ra. 8.8. mc. from 2d day	Dead 7 days later	Numerous..	33.7%	66.3%	Females, 32% without embryos
5592	None (control)	Killed 17 days later	One	.....	One	This female was immature
5593	Ra. 3 mc. from 6 to 10th day and 9.6 mc. from 10th day	Killed 17 days later	316	31 %	69 %	Worms large and apparently normal
Experiment 2						
5617	Ra. 11.8 mc. from 1st day	Dead 7 days later	Two	.....	Two	Neither contains embryos
5618	None (control)	Killed 7 days later	600±	36 %	64 %	Females, 1.5% without embryos
5619	None (control)	Killed 7 days later	800±	37 %	63 %	
5620	None (control)	Killed 7 days later	1,000±	41 %	59 %	
5621	Ra. 2 mc. from 8th day and 15.5 mc. from 11th day	Dead 16 days later	31	51.5%	48.5%	
5622	None (control)	Killed 18 days later	None	.....	.....	
5623	None (control)	Killed 18 days later	One	One	.....	
5624	None (control)	Killed 18 days later	Two	.....	Two	
5625	None (control)	Killed 18 days later	None	.....	.....	
5626	None (control)	Killed 18 days later	None	.....	.....	

EXPERIMENT 2

March 10, 1916. Ten normal rats were fed with equal amounts of trichinous muscle mixed with bread and milk. Radiation was commenced at once with Rat 5717, a tube of 11.8 mc. strength being used. This animal was found dead at the end of seven days. Another rat was radiated with a weak tube (2 mc.) from the eighth day and also with a stronger dose (15.5 mc.) from the eleventh day. It died 16 days after infection. The results obtained in both experiments are combined in the table on the preceding page.

While these results failed to demonstrate that radiation is of therapeutic value in the treatment of trichiniasis, they indicate that it appreciably modifies the development of the parasite in the intestine. Radiation after the females have become ripe, that is after the sixth day, fails to affect an earlier disappearance of trichinellae, or to produce distinguishable injury to them. In fact, the worms appear to persist longer and to be unusually large and well developed in the late radiated animals. That larvae had continued to be liberated from the female worms was shown by the presence of very small as well as partially developed worms in the striated muscles of these rats. Although the control rats killed seventeen and eighteen days after feeding on trichinous meat furnish few or no adult trichinellae in the intestine, numerous larvae were found in the skeletal muscles of all showing that infection had occurred.

Early radiation apparently had a greater effect on the development of trichinellae in the intestine. Radiation of the rat's abdomen from the second day after the ingestion of the cysts resulted in a retardation of development as shown by the number of immature females; 32 per cent of these showed no fully formed embryos as compared with 2 per cent in the control animals. Females were observed in the radiated rat which were so backward in their development that they were considerably smaller than normal males, although seven days had elapsed since their ingestion. There appeared to have been no general failure of the immature females to become inseminated, although this may have been accomplished later than normally. In only a few small undeveloped females was the receptaculum seminis not filled with spermatozoa. Subsequent experiments have shown that under normal or ordinary conditions the worms almost without exception are fully developed seven days, and usually six days, after they have entered the alimentary tract. Only a few larvae were found on careful search in the diaphragm of the rat radiated from the second day, whereas they were present in great numbers in the diaphragm of the control rat. Still earlier radiation, that is, from the time of the ingestion of the encysted larvae, appears to be even more effective, and in the animal in which this was carried out only two females, neither of which con-

tained embryos, were found. The three rats which served as controls for this animal each showed numerous well developed worms estimated at 600, 800, and 1,000, respectively.

Since the radiation employed was fatal to three of the four trichinous rats, the possibility that the injury to the host might indirectly affect the life of the parasites may be considered. That changes in the host resulting from radiation do not tend to destroy the worms is shown in the late radiated rat in which adult worms persisted longer than in the controls.

II. THE RADIATION OF ENCYSTED LARVAE BEFORE INGESTION

It appeared important, in order to estimate the dosage appropriate for the employment of shorter radium rays, to radiate the larvae before they were fed to the animals. For this purpose the filtration through the millimeter of silver was dispensed with and capillary glass tubes of emanation enclosed in steel tubes having walls 0.1 mm. in thickness were employed. Under-estimation of the effects of the short rays necessitated repeating the experiment several times. In Experiment 3, which is not presented in tabular form, none of the larvae in meat radiated with a 5.9 mc. tube for six and for three days developed when fed to mice. Control mice fed with untreated meat in every case showed trichinellae when killed later on.

For the purpose of making the observations more accurate, equal numbers of encysted larvae were fed to each animal in all subsequent experiments. The encysted larvae were radiated by wrapping strips of mouse diaphragm around the steel tube containing the emanation, in this way ensuring fairly uniform radiation of all portions of the muscle. The layer of muscle around the steel tube nowhere exceeded 1.5 mm. in thickness. The strips of diaphragm were subdivided when necessary so that an equal number of cysts could be fed to each mouse. This was accomplished by placing the bits of diaphragm in the mouth of the animal and holding the latter until the material was swallowed.

Experiment 4

April 14, 1916. Twelve mice were employed in this experiment. Two of these served as control animals, being fed each with 40 trichina cysts. The other ten were each fed 40 cysts which had been radiated for different periods with 5.5 mc. of emanation enclosed in a capillary glass tube and filtered through 0.1 mm. of steel. Tissue radiated was nowhere more than 1.5 mm. in thickness. Six of the animals were killed four and five days after this feeding, and a count made of the number of *Tr. spiralis* present in the small and large intestine of each. The other six were allowed to live for a longer period and the muscles were then examined to determine whether infection had taken place.

TABLE OF RESULTS—EXPERIMENT 4

No. Mouse	Larvae Radiated with 5.5 Mc.	Each Fed 40 Larvae	Killed	Mature ♂	Mature ♀	Immature ♂	Immature ♀	Total
5699	Control untreated	April 14	April 20	3	10	0	1	14
5700	Control untreated	April 14	April 21	0	10	0	0	10
5691	3 hrs. at 33 C.	April 14	April 20	0	0	0	0	0
5692	3 hrs. at 33 C.	April 14	April 20	0	0	0	0	0
5693	6 hrs. at 33 C.	April 14	April 20	0	0	0	0	0
5694	6 hrs. at 33 C.	April 14	April 21	0	0	0	0	0
5695	12 hrs. at 33 C.	April 14	May 4	Muscles negative				0
5696	12 hrs. at 33 C.	April 14	May 4	Muscles negative				0
5697	24 hrs. at 33 C.	April 14	May 4	Muscles negative				0
5698	24 hrs. at 33 C.	April 14	May 4	Muscles negative				0
5701	48 hrs. at 33 C.	April 14	May 4	Muscles negative				0
5702	48 hrs. at 33 C.	April 14	May 4	Muscles negative				0

Experiment 5

April 21, 1916. Fourteen mice were employed, two receiving untreated cysts, the others receiving equal numbers of cysts which had been radiated for different periods of time at room temperature. The technic employed was the same as that in the preceding experiment but shorter exposures were made. The radiated tissue was nowhere more than 1.2 mm. in thickness. The mice were all killed five or six days after the feeding.

TABLE OF RESULTS—EXPERIMENT 5

No. Mouse	Larvae Radiated with 7.1 Mc.	Each Fed 40 Larvae	Killed	Mature ♂	Mature ♀	Immature ♂	Immature ♀	Total
5738	Control untreated	April 21	April 26	0	3	0	1	4
5739	Control untreated	April 21	April 27	2	8	0	0	10
5726	2½ minutes	April 21	April 26	1	1	2	8	12
5727	2½ minutes	April 21	April 27	6	10	0	0	16
5728	5 minutes	April 21	April 26	3	5	3	2	13
5729	5 minutes	April 21	April 27	1	3	0	6	10
5730	10 minutes	April 21	April 26	1	8	2	4	15
5731	10 minutes	April 21	April 27	0	0	0	0	0
5732	20 minutes	April 21	April 26	0	0	0	0	0
5733	20 minutes	April 21	April 27	7	12	0	0	19
5734	40 minutes	April 21	April 26	0	0	0	0	0
5735	40 minutes	April 21	April 27	1	1	0	0	2
5736	80 minutes	April 21	April 26	0	0	0	0	0
5737	80 minutes	April 21	April 27	0	0	0	0	0

It is apparent from the above experiment that radiation for eighty minutes with 7.1 mc. is fatal to the encysted larvae, but the results obtained for the next shorter periods are rather variable, one positive and one negative result being obtained in each of the three successive periods. Radiation for two and one-half and for five minutes appears not to have been markedly injurious. It was thought possible that since certain portions in the length of the steel tube employed were less

radioactive than others, certain portions of the diaphragm exposed may have been subjected to less radiation accounting for the irregularity of the results obtained. On account of this it was considered necessary to repeat this experiment, paying especial attention to the equal radiation of all parts of the material used.

EXPERIMENT 6

May 1, 1916. Ten mice were employed, two served as controls, and the others received equal numbers of encysted larvae radiated for various periods. These were killed four and five days after this feeding and the intestine examined for *Tr. spiralis*.

TABLE OF RESULTS—EXPERIMENT 6

No. Mouse	Larvae Radiated with 6.6 Mc.	Each Fed 40 Larvae	Killed	Mature ♂	Mature ♀	Immature ♂	Immature ♀	Total
5749	Control untreated	May 1	May 5	4	0	0	1	5
5750	Control untreated	May 1	May 6	1	3	0	8	12
5747	20 minutes	May 1	May 5	1	0	2	5	8
5748	20 minutes	May 1	May 6	0	0	0	1	1
5745	30 minutes	May 1	May 5	0	0	0	0	0
5746	30 minutes	May 1	May 6	0	0	0	0	0
5743	40 minutes	May 1	May 5	0	0	0	0	0
5744	40 minutes	May 1	May 6	0	0	0	0	0
5741	60 minutes	May 1	May 5	0	0	0	0	0
5742	60 minutes	May 1	May 6	0	0	0	0	0

From the three preceding experiments the lethal dosage of radiation for encysted trichinae is determined. They are made non-infectious for mice by radiation with 6.6 mc. filtered through thin glass and 0.1 mm. of steel in an exposure of thirty minutes. The cysts exposed were at a distance of not over 1.5 mm. from the source of radiation. It would be of interest to learn more concerning the effects of this amount of radiation on encysted larvae whether they are killed outright, or the cyst made more resistant to the digestive juices or the larvae injured to such an extent that they are passed from the alimentary tract before they can recover sufficiently to maintain their existence. In all of the present experiments only the immediate result of radiation as shown by the absence or by the arrested development of worms, has been determined. It would be of considerable interest to note whether any remote or late changes are brought about, but this probably would be more readily determined in a free-living rather than in a parasitic species.

III. THE DIRECT RADIATION OF TRICHINELLA SPIRALIS FROM THE INTERIOR OF THE ALIMENTARY TRACT

Since the short rays were found to be effective in the destruction of the larvae the direct radiation of the worms from the interior of the intestine was next undertaken. Through radiating the interior of

the intestine by means of tubes of emanation fed to the animal it was hoped to utilize very short rays. The movement of the intestinal contents was expected to prevent undue burning of the mucosa of the small intestine and the incorporation of the tube in more or less solid fecal material was hoped to protect the wall of the large intestine from serious injury. Minute tubes of emanation were prepared by Doctor Duane. Since these measured only from 2 to 3 mm. in length and a fraction of a millimeter in diameter, they could be readily introduced into the stomach of the mouse. In order to accomplish this, a large syringe needle, the point of which had been ground off square, covered with paraffin and dipped in oil so that it could be readily passed down the esophagus of the mouse, was used. The emanation tube having been placed in the needle, it was forced by a plunger into the stomach of the mouse. It could readily be determined at any time whether the tube had been passed from the intestine or was still in the body of the mouse by placing the latter on the ionization chamber of the measuring apparatus.

EXPERIMENT 7

June 27, 1916. Nine mice were each fed 40 trichina cysts in bits of mouse diaphragm. Minute glass tubes of emanation were introduced into the stomach of three of these, one receiving a tube on the first day, another on the second day, and another on the third day.

TABLE OF RESULTS—EXPERIMENT 7

No.	No. Fed	Radium Fed	Died	No. Tr. Spiralis
5803	80 cysts	3.1 mc. 7 hours later	Killed 5 days	24 (4 immature)
5804	80 cysts	4.1 mc. 28 hours later	Dead 6 days	57
5805	80 cysts	3.2 mc. 51.5 hours later	Dead 6 days	46
5806	80 cysts	Control	Killed 5 days	33
5807	80 cysts	Control	Not examined	
5808	80 cysts	Control	Not examined	
5809	80 cysts	Control	Not examined	
5810	80 cysts	Control	Not examined	
5811	80 cysts	Control	Not examined	

In the first mouse (5803) the tube failed to pass from the intestine during the four days and fourteen hours which elapsed before it was killed on account of its weak appearance. The tube fed the second mouse (5804) was still in its body after three days, but had been passed when found dead two days later. The tube fed the third animal (5805) was passed within forty-eight hours. Notwithstanding the small dosage, 3.2 mc., for so short a period, this animal died before the end of four days after its introduction. This mouse presented a reddened area in the wall of the large intestine. The others showed no local effects of the radiation, but all showed a striking shrinkage in the size of the spleen so characteristic of radiated animals. These results show unmistakably that in the mouse even fatal doses of radium emanation acting

from the interior of the intestine fail to prevent the development of *Tr. spiralis*. It is quite apparent that this parasite is not especially vulnerable to rays which have an immediate destructive influence on the lymphoid tissue of the host.

The results of the foregoing experiments are thus rather discouraging with respect to the application of radiation to parasitic worms. It is rather remarkable that *Tr. spiralis* is so little affected after commencing its development within the body when it, in its encysted state, is so quickly destroyed by radiation outside the body. Whether internal radiation would accomplish more in larger, more resistant animals remains to be determined. The acceleration of the passage of radium emanation through the large intestine by the employment of a cathartic might be of value, but was not tried in these experiments. It would appear, however, that, under the conditions of the above experiment, radiation destroys the resistance of the animals before the parasite is markedly affected.

These results do not, therefore, furnish an experimental basis for the treatment of schistosomiasis by radiation from the interior of the bladder. Although this treatment would for some reasons appear especially applicable to this disease, it would probably be impossible, with the short rays, to reach all the adult worms, for they are frequently situated at a considerable distance from the surface of the mucosa. Treatment of this disease by radiation, although justified on theoretical grounds, must for the present be regarded as experimental in character, and, even if no untoward results occur, it will be difficult to determine just what has been accomplished. On the other hand, the localization of the worms and the relatively large size of the host are distinctly in favor of this form of treatment. The fact that it is a disease frequently attended with serious complications would appear to warrant cautious treatment by radiation, provided that the experimental nature of the treatment is explained to the patient, and provided that changes in the local condition may be followed by cystoscopic examination and by observations with reference to the number of ova discharged in the urine.

SUMMARY

By radium radiation from the surface of the abdomen of the rat the injury of fully developed *Trichinella spiralis* has not been accomplished. These worms appeared well developed and persisted longer than in controls.

Similar treatment from the second day after the ingestion of cysts has apparently resulted in a retardation of development, 30 per cent of the females being immature.

In a rat radiated in this manner from the time it was fed trichinous meat, only two immature worms were found seven days later, indicating that radiation of the larvae before they have entered upon their period of development free in the intestine is fatal to them.

The radiation of encysted larvae with 6.6 mc. of emanation, 0.1 mm. steel filtration, at a distance of not over 1.5 mm. for thirty minutes renders them non-infectious for mice.

Radiation from the interior of the intestine—employing emanation in minute glass tubes for the utilization of short rays—in amounts sufficient to cause the death of the mice employed has not prevented the development of *Tr. spiralis*.

These results fail to furnish an experimental basis for the treatment of schistosomiasis by radiation, so that if the latter is attempted it should be regarded as an experiment rather than an approved mode of procedure.

Observations on the life history of *Trichinella spiralis* made in the course of these experiments indicate that certain points emphasized in books of reference do not apply to the development of this parasite in rats and mice.

Trichinella spiralis is found only in small numbers in the duodenum and jejunum of rats and mice which show great numbers in the lower portion of the small intestine. It is also occasionally found in the cecum and large intestine.

The life of this parasite is comparatively short in the rat, and it is found to have disappeared or is present only in small numbers eighteen days after infection.

No evidence has been obtained that the males disappear early in the infection. A sex ratio of 1 ♂ : 2 ♀ observed six and seven days after infection has shown no marked change for the ten days following. A male *Trichinella* has been found in a rat from which all females had diseappeared.

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NOTES ON SOME NEMATODES FROM FRESH-WATER FISHES *

HENRY B. WARD AND THOMAS B. MAGATH

The parasitic nematodes are of conspicuous importance in the field of human disease and also in diseases of the domestic animals, and in his treatise on fish diseases, Hofer (1906), discussing the significance in fish culture of parasites and parasitic diseases, states that among them the nematodes outrank all others in number of types. Yet as fish parasites these forms are almost unknown in North America, and references to them are confined to a few brief notes, almost all of which came from the pen of the distinguished Philadelphia microscopist, anatomist, and parasitologist, Joseph Leidy, whose pioneer work published between 1850 and 1886 includes many records of great value on this group.

In this little-explored field the senior author has been making observations for many years and in collaboration with the junior author was led recently to undertake an extended study of nematode parasites from North American fresh-water fishes which has yielded a number of new and interesting forms; these are briefly described here in advance of the appearance of the complete article in which will be given fuller data on the structure and relationships of these species. Especial thanks are due the United States Bureau of Fisheries for aid in securing material.

It is interesting to note that among the eight forms described as new species, three fall within new genera and five agree sufficiently with European forms to be listed in already existing genera. Seven out of the nine forms described in this paper come within the limits of the Spiruroidea, so that this superfamily appears to hold a prominent place among parasites of fresh-water fishes.

Cystidicola Fischer von Waldheim 1797. — The type species *C. farionis* from the air-bladder of the trout and other fishes is a common form in Europe; it has also been reported from the lake trout of Lake Erie. Leidy (1886) described a parasite of the air-bladder of the lake trout as *Filaria stigmatura*. We have the same parasite from the white fish, lake trout, and lake herring in Lake Erie, Lake St. Clair and Lake Michigan. It is clearly not a Filaria, but belongs to the genus Cystidicola, and should be called *C. stigmatura*. Two small

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uncinate lips, a short buccal capsule or tubular pharynx followed by a long esophagus divided into an anterior muscular and a posterior glandular region, indicate the position of this form in the superfamily Spiruroidea created by Railliet and Henry (1916) and in the family Camallanidae established by the same authors.

Camallanus Railliet and Henry 1915. — Among the commonest and best known parasites of fresh-water fish in Europe is the so-called Hooded Worm, easily recognized by its horny oral armature in the form of heavy ribbed valve-shaped lips of dark brown material with corner anchors of trident form. More than fifty names have been given these worms which most often have been assigned to the genera Dacnitis and Cucullanus, but have recently been definitely located in a new genus, Camallanus, by Railliet and Henry (1915a). Though in habit hookworms by virtue of their persistent attachment to the intestinal wall, they do not possess a buccal capsule like all true hookworms; the valve-shaped lips form powerful lateral jaws that grasp the tissue, whereas the true hookworms possess a hollow cup into which the tissue is drawn by suction. Two species have been found in North American fresh-water fishes. The first is

Camallanus ancylodirus nov. spec. — The head (Fig. 4) is bent sharply ventrad, whence the specific name. The mature female is 25 mm. long by 0.56 mm. in maximum diameter. The caudal tip is bluntly conical, and 0.45 mm. in front of it lies the anus. The vulva is three-fifths of the length from the anterior end. The trident in the oral armature has three or rarely four prongs, irregular in form and 0.21 mm. long. The lips measure 0.142 to 0.168 mm. long by 0.18 to 0.187 mm. broad. The uterus, loaded with minute embryos, fills the entire body save at the extreme ends. The anterior region of the esophagus is 1.416 mm. long and 0.096 to 0.15 mm. broad; the posterior region measures 1.368 by 0.072 mm.

Mature males measure 15 mm. in length by 0.38 mm. in breadth at the center of the body. The lips are 0.126 mm. long by 0.12 mm. wide, and the trident arms 0.18 mm. long. The anus (Fig. 10) lies 0.156 mm. from the extreme tip. The caudal alae are 0.92 mm. long. The two spicules (Fig. 11) are nearly equal in length, but one is only half as heavy as the other. The posterior end is rolled in a half circle and the number of the caudal papillae could not be determined.

This parasite came from the intestine of the German carp at Fairport, Iowa, and is the first reported from that host on this continent. Evidently it is a native species that has accommodated itself to this new host, and its original host is as yet unknown. Instances are infrequent in which a host is known to have acquired an entirely new parasite.

Camallanus oxycephalus nov. spec. — Only females were found. Length up to 25 mm. and maximum breadth 0.27 mm. The anterior end (Fig. 3) is perfectly straight and much smaller than the preceding species. The body tapers regularly from the center about equally in both directions. A small but distinct constriction, or "neck," lies just behind the oral tridents and conforms to the curvature of the trident branches. The muscular esophagus is 0.47 mm. long and 0.085 mm. in diameter at the narrowest point, expanding near the end to 0.105 mm.; the second region of the esophagus is 0.57 mm. long, of nearly equal diameter throughout and as broad as the maximum breadth of the muscular esophagus. The vulva is located at the anterior margin of the middle third of the body. This species was taken from the intestine of the white bass and of the black crappie at Fairport, Iowa.

Cucullanus O. F. Müller 1777. — As Railliet and Henry have shown recently (1915a), the forms which rightly belong here are those to which the name Dacnitis Duj. 1845 has often been applied. They are characterized as follows:

Anterior end bent dorsad. Mouth elliptical with major axis dorso-ventral, bounded by two lateral valves recalling those of Camallanus. Esophagus pestle-shaped without bulb. Males without caudal alae; two equal spicules and an accessory piece. Preanal sucker without chitinous ring. Female with vulva near center. In alimentary canal of fish.

The genus was placed in the Heterakidae by Railliet and Henry chiefly because of the preanal sucker of the male. However, a ventral sucker is by no means confined to this family, and this one has no marginal ring such as is present in other Heterakidae; finally, the three lips of the Heterakidae are wanting. Accordingly, we have placed the genus in the Spiruroidea with which it agrees in the lateral valves at the mouth and the double esophagus, both characteristic of that group. The genus is the sole known representative of a family, the Cucullanidae, which differs from all other forms in the Spiruroidea through the possession of the ventral sucker in the male.

Cucullanus clitellarius nov. spec.—Body generally uniform in diameter, except for clitellar-like swelling 1.5 mm. from anterior tip (Fig. 5). Head bent dorsad 60 to 90 degrees. On each oral margin three papillae. Oral valves 0.45 by 0.32 mm. in female, and 0.33 by 0.24 mm. in male.

Males 10 to 11 mm. long by 0.38 mm. broad. Esophagus 1.45 mm. long and 0.12 to 0.22 mm. wide. Caudal region (Fig. 9) bent in a single turn. Ventral sucker 0.51 mm. anterior to anus, 0.1 mm. in diameter. From anus to tip of body 0.39 mm. Spicules 1.62 mm. long,

0.035 mm. broad, shaped like a gouge; accessory piece dagger-shaped, 0.06 long by 0.015 mm. broad. Two small papiliae just in front of anus; four pairs of postanal papillae, of which two pairs are large, rounded, and only 0.012 mm. from extreme tip.

Females 12 to 17 mm. long, 0.5 mm. broad. Esophagus 1.6 mm. long and 0.13 to 0.32 mm. wide. Distance from anterior end to vulva from five-ninths to two-thirds total length. Uterus and ovary double. Ova 63 by 46μ.

Parasitic in intestine of lake sturgeon (*Acipenser rubicundus*) in Lake St. Clair.

This genus has been heretofore the sole representative of its family, but among parasites in fresh-water fishes we have met another type that is sufficiently related to fall within the limits of the family, and yet cannot be brought within the limits of the same genus. To receive it a new genus has accordingly been created with the following characteristics:

Dacnitoides nov. gen.—Much like Cucullanus, except that head is not flexed and body is straight; spicules lack accessory piece, and only a single ovary is developed. A well developed intestinal cecum is present.

Dacnitoides cotylophora nov. spec.—Males 4 to 6 mm. long and 0.2 mm. broad. Mouth dorso-ventral, bounded by lateral valves. Cuticular ridge with three papillae at extreme anterior margin of each valve. Esophagus 0.5 to 0.6 mm. long, 0.06 to 0.12 mm. broad, distinctly divided into two regions; anterior region 0.2 mm. long. Intestine large, provided with dorsal cecum extending anteriad to junction between two regions of esophagus. Ventral sucker (Fig. 7) 0.41 mm. in front of anus, which is 0.12 mm. from posterior tip. Spicules 0.89 mm. long and only 5μ broad. Caudal papillae: one pair on anterior margin of sucker, four pairs between sucker and anus, four pairs of postanal papillae and one single median papilla immediately in front of anus.

Females 4 to 5.5 mm. long by 0.28 mm. in width at vulva; body distinctly short and heavy. Anterior end (Fig. 6) rounded, posterior end acutely pointed. Anus 0.14 mm. from posterior tip, with four slender spines halfway between. Vulva about five-eighths of total length from anterior end. Anterior and posterior uterine branches, but latter terminates blindly, and only former has an ovarian tube at end. Eggs measure 65 by 40μ, and contain embryos in early clearage stages.

This parasite was common at Lake St. Clair in the intestine of the yellow perch (*Perca flavescens*), and more rarely of the wall-eye (*Stizostedion vitreum*).

In that this species possesses an intestinal cecum it resembles the family of the Heterocheilidae which Railliet and Henry established

among the Ascaroidea on the basis of the development of such an organ. In other anatomical features it departs as widely from that family as it does from the Heterakidae.

An interesting parasite was taken from the intestine of the bowfin, or fresh-water dog-fish, *Amia calva,* both in Lake St. Clair, Michigan, and at Fairport, Iowa. It is of a very generalized character and hence difficult to define except in negative characters, or to locate in the system. While we are inclined to place it in the Spiruroidea under the family Spiruridae, we must confess that this decision is open to criticism, and it may have to be made the representative of a new family standing half way between the Ascaroidea and the Spiruroidea. It must certainly be made a new genus characterized as follows.

Haplonema nov. gen.—Body rather robust, but not large; anterior end bent or coiled, without lips or papillae, but with lateral alae ("wings"). No buccal capsule; esophagus muscular, without bulb, divided into two regions by partition near center. Posterior end of female straight, or slightly curved behind anus, with two minute papillae. Posterior end of male without bursa or alae, with two equal spicules of moderate length. Two pairs of preanal papillae and three pairs of postanals. Ovary laid in transverse loops ventral to intestine in both anterior and posterior regions. Uterus with anterior and posterior branches, vulva near center of body. Oviparous.

Haplonema immutatum nov. spec. — Males less frequent than females, measure about 9.5 mm. in length by 0.2 mm. in breadth anteriorly and 0.18 mm. posteriorly. Females about 15 mm. long by 0.31 mm. in maximum breadth in the anterior region. Vulva five-eighths of length from anterior tip.

Anterior end (Fig. 1) bent in a half circle with lateral cuticular folds extending back 2.5 or 3 mm. from anterior tip. Esophagus prominent, muscular, 0.65 mm. long in male and 0.8 mm. in female; width of esophagus, 0.06 mm. anteriorly and 0.1 mm. near its posterior end. Esophageal partition inconspicuous, near center of length; two regions alike in structure.

Spicules (Fig. 2) two, equal, flat, ribbon-like, 0.75 mm. long, by 0.02 mm. wide. Eggs abundant, with moderately thick, smooth shells; average size 65 by 45μ.

The genus Spinitectus was established by Fourment in 1883 to contain parasites of fishes characterized by circles of retrorse spines on the posterior margins of transverse rings. To the four species known in Europe we add a new form common in some places here.

Spinitectus gracilis nov. spec.—Mature females 11 to 19 mm. long; body divided more or less distinctly into slender transparent anterior region about 6 mm. long and 0.066 mm. wide, and larger, darker, more

opaque posterior region about 12 mm. long and 0.14 mm. broad, crowded with internal organs, especially the uterus gorged with eggs. Tail abruptly conical, 0.096 mm. from anus to tip in female, only 0.066 in male. Vulva three-fourths length of body from anterior end, inconspicuous.

Spinous rings begin 0.12 mm. from anterior tip. First 6 to 8 rows more prominent than those later. Subsequent rows (up to 28 or 30) smaller, closer, with spines much lighter and shorter; last rows difficult to detect. Total about 130 rows. Rings about 0.03 mm. apart at anterior end, and contain 40 to 50 spines in each circle. Largest spines 8μ long, smallest less than half as long.

Male 12 mm. long by 0.042 mm. in diameter in anterior region and 0.75 mm. at widest point. Posterior end coiled in spiral with 2 to 3 turns. Narrow lateral alae 0.33 mm. long, near caudal tip, not supported by papillae or ribs. On ventral surface, 1 mm. anterior to anus a series of 4 to 8 longitudinal rows of small ridges, each about 5μ long and 3μ high. Spicules very heavy, longer scoop-shaped; shorter, arcuate, oblique, probably not protrusible.

Pharynx tubular or funnel-shaped, short. Muscular esophagus narrow, 0.33 mm. long in female and 0.25 mm. in male, no marked boundary between it and glandular region. Egg-filled uterus large. Ova 41 by 24μ, with thick transparent wall and without polar processes.

Some specimens 4 to 5 mm. long by 0.35 mm. broad have spines extending even to the anal region, 175 rows in all. From the vulva a broad vagina projects anteriad bearing at its inner end anterior and posterior uterine branches.

This parasite occurs in the alimentary canal of the black crappie, sheepshead, and white bass at Fairport, Iowa. In life it is transparent, and the spinous rings are very distinct. The worms are not attached to the wall, but lie free in the lumen of the gut. The spines are encased in masses of food particles.

Railliet and Henry include in this genus as *Spinitectus cristatus* a form described by Linton from the hake as *Filaria serrata*. They use his description of the male for the genus since Fourment found no males in his material. Our form differs from Linton's in the absence here of papillae he described and in the presence here of caudal alae he neither mentions nor figures.

A nematode parasitic in the perch in Lake St. Clair is assigned without hesitation to the genus Ichthyonema. The genus is closely related to *Dracunculus medinensis* of man. In Europe various species are abundant, and widely distributed, but one has never been reported before this in North America. Possibly some of the worms listed as "Filaria" from American fishes really belong here. The species we have does not agree with any known form and is designated as

Ichthyonema cylindraceum nov. spec. — Male unknown, probably minute. Mature female 100 mm. long, of nearly equal diameter (0.48 mm.) everywhere. No lips or papillae. Esophagus 1.09 mm. long, 0.066 mm. in diameter. Vulva and vagina atrophied, no vestiges discernible. Uterus crowded with undeveloped ova (i. e., female unimpregnated), ova almost spherical, measure 0.044 mm. in diameter.

In abdominal cavity of *Perca flavescens,* Lake St. Clair.

The worm was delicate, semitransparent, and very fragile owing to the thin body wall. The lateral lines are broad, light colored, and conspicuous. In Europe almost half of the females found are like our material, unimpregnated owing apparently to scarcity of males. This species is of great interest from its relationship to the Guinea Worm of man.

Among the Ascaridae, Railliet and Henry (1915) have grouped those forms having either an intestinal or an esophageal cecum into a single family, the Heterocheilidae. One form we have studied falls within this group, but cannot be placed in any of the genera found in it, hence a new genus is created to contain it characterized as follows:

Hysterothylacium nov. gen.—Body without anterior tunic, but with narrow lateral alae ("wings"). Lips three, not prominent. Esophagus long, slender, with terminal spherical bulb. Intestine with short simple cecum, arising from anterior end of intestine, directed posteriad. Males with two equal curved spicules, papillae (?). Females unknown.

Hysterothylacium brachyurum nov. spec.—Length of male 32 mm., maximum width just behind center of body, 0.66 mm. Lateral fin (Fig. 8) from base of lip to esophageal bulb or further; width one quarter the diameter of body.

Esophagus 3.1 mm. long, 0.1 to 0.13 mm. wide; bulb with three teeth, cecum 0.94 mm. long, 0.08 mm. wide. Spicules equal, 0.72 mm. long by 0.045 mm. wide. Pyriform sperm-vesicle prominent. In stomach of black bass, Lake St. Clair, Michigan.

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EXPLANATION OF PLATE

Fig. 1.—*Haplonema immutatum;* head of female showing lateral fin fold, excretory pore, two regions of esophagus, esophageal valve, and intestine. × 72.

Fig. 2.—*Haplonema immutatum;* tail of male showing spicules and papillae. × 72.

Fig. 3.—*Camallanus oxycephalus;* head of female showing valves, trident, ring, and anterior region of esophagus. × 72.

Fig. 4.—*Camallanus ancylodirus;* head of female showing oral armature and two regions of esophagus. × 22.

Fig. 5.—*Cucullanus clitellarius;* head of female showing valves, double esophagus, esophageal valve and intestine; also half of clitellar-like swelling. × 20.

Fig. 6.—*Dacnitoides cotylophora;* head of female, showing oral armature, esophageal regions, intestine, cecum, and anterior coils of ovary. × 70.

Fig. 7.—*Dacnitoides cotylophora;* tail of male, showing sucker, spicules and papillae. × 70.

Fig. 8.—*Hysterothylacium brachyurum;* head of male showing lips, lateral fin-fold, esophagus, esophageal bulb, intestine, and cecum. × 22.

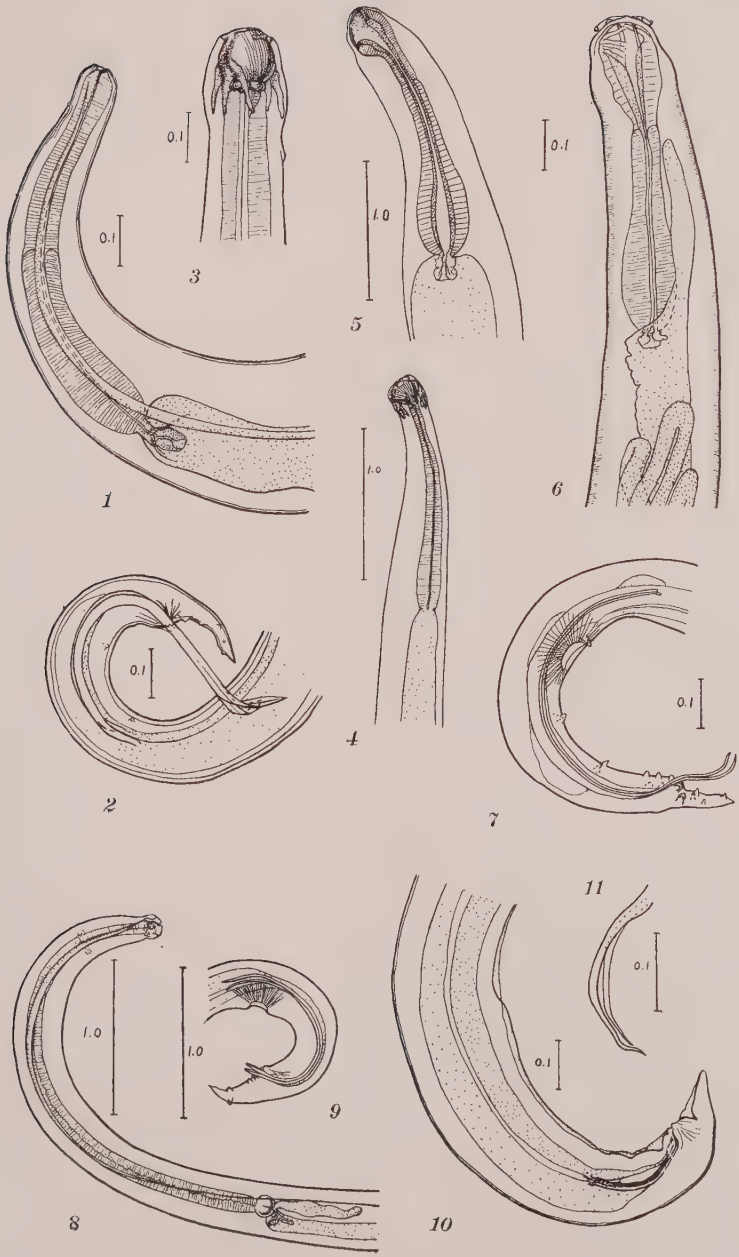
Fig. 9.—*Cucullanus clitellarius;* tail of male, showing sucker, spicules and papillae. × 22.

Fig. 10.—*Camallanus ancylodirus,* tail of male showing spicules and papillae. × 70.

Fig. 11.—*Camallanus ancylodirus,* spicules of male. × 110.

All reference lines in millimeters or tenths as indicated on plate.

PLATE



OBSERVATIONS ON POLYCYSTID GREGARINES FROM ARTHROPODA*

MINNIE E. WATSON

The following pages contain observations on already known and new species obtained by the writer chiefly during the past summer.

BULBOCEPHALUS WARDI *nov. gen., nov. spec.*
[Figures 1, 2, and 3]
Host: Clerid larva (Det. E. P. Felt)
Habitat: Intestine
Location: Oyster Bay, Long Island, June, 1916

Fifty or more specimens of this gregarine, mostly free trophozoites, were taken from a single Clerid larva. The sporonts are solitary and rather small, and the species is characterized chiefly by the distinct bulb in the mid-region of the large epimerite of the trophozoite.

The longest sporont seen measured 190μ in length and 45μ in width. One trophozoite exceeded in length the longest sporont, being 290μ in length. The protomerite of the sporont is slightly longer than wide, broadly rounded in front, and slightly constricted at the septum. The deutomerite is widest at the "shoulder" and tapers from thence, ending in a long blunt-pointed extremity. The average ratio of length of protomerite to total length is about 1:5; the protomerite and deutomerite are of approximately the same width.

The epimerite is unique. It consists of three parts: *a*, a broad base fitting on the apex of the protomerite, tapering at the top to form a neck; *b*, a spherical bulb, in the middle; *c*, a stout style at the apex. In other words, the epimerite consists of a stout broad-based style, bulbous in the middle. In length, it measures one-fifth to one-third the total length of the trophozoite. Complete dimensions of several epimerites are given in the table at the end of this species.

The protoplasm is dense, rendering it dark in color in both protomerite and deutomerite. The ellipsoidal nucleus is not visible in the live sporont; when stained, it is seen to be small in the sporont (larger in comparison in the trophozoite), and lies diagonally across the largest part of the deutomerite. One karyosome is present.

The writer is unable to classify this species in any known genus. The epimerite is most nearly related to that of the genus Stylocephalus; in this genus it consists of a more or less long, slender neck terminating

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in a large or small papilla. There may or may not be a bulbous base. The nucleus is ellipsoidal. The type species of the genus is *Stylocephalus oblongatus* (Hammerschmidt) Watson, see Watson 1916: 159.

The present specimens differ from the type species of the aforesaid genus in but one respect: The bulbous papilla of the epimerite is placed not at the apex of the cylindrical neck, but at its mid-point. In no species of the named genus is there a papilla at other than the apex. It therefore devolves upon the writer to describe a new genus in order to place the specimens found. I therefore designate it Bulbocephalus, and place it as follows:

Family Stylocephalidae Ellis. Sporonts solitary, epimerites varied. Nucleus ovoidal. Dehiscence by pseudocyst. Spores irregularly shaped, brown or black, in chains (Watson 1916: 47).

Type Genus Stylocephalus Ellis. Epimerite a dilated papilla at the end of a long slender neck. Cyst covered with small papillae and indentations. Spores hat-shaped.

New Genus Bulbocephalus. Epimerite a dilated papilla placed in the middle of a rather long slender neck. Cyst and spores unknown.

The species is named for Professor Henry B. Ward of the University of Illinois.

A table of typical measurements, in microns, is given herewith:

Length epimerite	60	x	x	40	30
Length protomerite (without epimerite)	50	30	40	15	20
Length deutomerite	180	62	30	55	40
Total length sporont	290	192	170	110	90
Width epimerite	16	x	x	16	10
Width protomerite	42	32	40	20	18
Width deutomerite	37	45	40	28	31
Ratio length prot. (without epim.): total length	1:5.8	1:6.4	1:4.2	1:7	1:4.5
Ratio width prot.: width deut.	1.1:1	1:1.4	1:1	1:1.4	1:1.7
Diameter nucleus	.....	.....	8x15	.....	11x27

BULBOCEPHALUS ELONGATUS *nov. spec.*

[Figures 4, 5, and 6]

Host: Cucujus larva (Det. Adam Boving)
Habitat: Intestine
Location: Oyster Bay, Long Island, August, 1916

Specimens of this peculiar gregarine were very abundant in the one beetle larva obtained. The sporonts are relatively very long and slender, unlike those of any gregarine before taken by the writer. The maximum length recorded for a sporont is 600μ; the maximum width, 50μ. The ratio of length of protomerite to total length is 1: 11, and the ratio of width of protomerite to width of deutomerite about 1:1.

The protomerite is widest near the septum, at which there is a slight constriction, and the deutomerite is widest at the "shoulder," gradually tapering from thence to form a very long posterior extremity. The protoplasm is dense in the sporont, but much less so at the posterior

end, and the nucleus is obscured in the adult. In the trophozoite, it is seen to be small and ellipsoidal and placed diagonally to the long axis of the animal.

The epimerite is a rather long, stout style dilated in the middle to form a good sized papilla. The shape of the epimerite indicates that this species is closely allied to the preceding, and is therefore placed in the new genus, Bulbocephalus.

A table of a few measurements in microns follows:

Length epimerite	x	x	40
Length protomerite (without epimerite)	47	30	20
Length deutomerite	553	300	170
Total length sporont	600	330	230
Width epimerite	x	x	10
Width protomerite	35	40	20
Width deutomerite	30	50	23
Ratio length prot.: total length	1:13	1:11	1:11
Ratio width prot.: width deut	1.1:1	1:1.2	1:1.1
Diameter nucleus	.....	.....	5x11

PYXINIA BULBIFERA *nov. spec.*

[Figures 7, 8, 9, 10, 11 and 12]

Host: *Dermestes lardarius* Linn. (Det. E. P. Felt)
Region of infection: Mid-intestine
Location: Oyster Bay, Long Island, May, 1916

Fifty or more specimens of this parasite were taken from one adult larder beetle, many of them being free trophozoites.

The sporonts are solitary and long and slender in shape, except at the "shoulder," where they are appreciably wider (Figs. 7 and 8). The largest sporont seen measured 850μ long and 160μ wide; the smallest specimen without an epimerite, 600μ long. The average ratio of length of protomerite to total length of sporont is 1:5, and the ratio of width of protomerite to width of deutomerite is 1:1.3.

The protomerite is generally of the same width as height, infrequently a little wider than high. It is bluntly cone-shaped, the widest portion lying two-thirds the distance from the apex, being constricted below this to meet the septum. There is often a slight indentation at the apex, left by the dropping off of the epimerite. The epicyte forms a very thick layer over the end of the protomerite (Fig. 9).

The epimerite consists of two parts, a stout bulbous crenulate and crateriform base and a short thick style. The bulbous base was frequently seen to expand and contract with considerable force by means of longitudinal folds which open and collapse, the motion of the myonemes probably forcing protoplasm in and out of the epimerite from the protomerite (Figs. 10, 11 and 12). The epimerite measures from 60μ to 100μ in length on large free trophozoites. The largest epimerite seen measured as follows: Style, 70μ long and 10μ wide at its base; bulb 30μ high and 35μ wide at its widest part.

The deutomerite tapers gradually from the "shoulder," ending in a blunt point.

The protoplasm is dense in most parts of the body. The widest portion of the deutomerite is very dark brown, nearly black, the posterior end being lighter in color. The protoplasm gradually becomes less dense in the latter region and the granules cease entirely some distance before the end is reached. The protomerite is tan in color and the epimerite transparent.

The nucleus is scarcely visible in an unstained sporont, but in stained specimens is seen to be an ellipsoidal body lying always at right angles to the main axis of the body and never diagonally, as is often the case in gregarines. A single large karyosome is present. The nucleus measured in a trophozoite about 90 by 40μ.

Myonemes were visible at the end of the deutomerite and in the protomerite with a magnification of 490; longitudinal striations were seen with a magnification of 770 and intense transmitted light. Brownian movement was noted where the protoplasm was least dense, viz., at the apex of the protomerite and the tip of the deutomerite.

Neither cysts nor spores were seen.

Although species of the genus Pyxinia have been described from the genus Dermestes, the present specimens do not fit any of these descriptions. They differ from *Pyxinia rubecula* Hammerschmidt (Léger, 1892: 140) chiefly in shape of the epimerite, which in the latter species is urn-shaped with a wide mouth and a crenulate periphery, and with a short style. Léger's figure for an epimerite of this species indicates the diameter of the urn to be five times its depth and the minute style so short that it scarcely projects beyond the rim. The present specimens indicate an urn narrower at the periphery than elsewhere and a style nearly as wide at its base as the encircling crenulate rim. In shape and proportions, the two species compare favorably, and the host is a beetle of the same genus, although previously described from Europe.

The conspicuous refractile pyxinin crystals of *P. crystalligera* Frenzel (1892: 314) are lacking in the present species, and the long, slender sporonts with bulbous protomerites contrast strongly with the species described above. Maximum length of sporonts and shape of the epimerite are similar in the two.

The other two described species of Pyxinia, *P. frenzeli* Laveran and Mesnil, and *P. möbuszi* Léger and Duboscq (Watson, 1916: 151) are radically different from this species in size and shape of the sporonts and in the character of the epimerite.

Dimensions of a number of typical specimens are appended herewith, measurements being in microns:

Length epimerite	x	x	40	x	100
Length protomerite (without epimerite)	150	150	150	160	140
Length deutomerite	700	690	600	580	500
Total length	850	840	750	740	640
Width protomerite	150	150	140	110	120
Width deutomerite	160	200	190	150	150
Ratio length prot. (without epim.): total length	1:5.6	1:5.6	1:5	1:4.6	1:4.6
Ratio width prot.: width deut	1:1	1:1.3	1:1.3	1:1.3	1:1.2
Diameter nucleus	.....	.....	.....	.....	90x40

GREGARINA NEGLECTA *nov. spec.*

[Figures 13, 14, 15, and 16]

Host: *Ceuthophilus neglectus* Scudder (Det. A. N. Caudell)
Habitat: Intestine
Location: Oyster Bay, Long Island, August, 1916

Four associations of this gregarine were found in the mid-intestine of one specimen of this camel cricket. Although very similar to many other species of the genus Gregarina in shape, this species may be characterized by a papillate protomerite in the primite and a perfectly egg-shaped satellite, there being no constriction at the septum.

The associations seen were all of about the same length, the maximum length of an association being 900μ; that for a single sporont 500μ. The maximum width measured was 230μ. In every instance, the primite was longer than the satellite, maximum length of the satellite being 430μ. The average ratio of length of protomerite to total length of the primite is 1:6; for the satellite the average is about 1:8.

The primite is elongate-ellipsoidal in shape and the satellite ovate, widest anteriad. The protomerite of the primite is broadly rounded, widest at the base and papillate at the apex; it is approximately one and two-thirds times as wide as high. There is a slight constriction at the septum. The deutomerite is regularly ellipsoidal, terminating in a broadly rounded extremity.

The protomerite of the satellite is very broadly rounded anteriorly, but with a trace of the papilla so prominent in the primite. It is three times as wide as high and is widest at the septum; there is no constriction at the septum. The deutomerite is widest near the middle and terminates in an extremity less well rounded than in the primite.

This species is very dark brown in color with the protomerite slightly less dense. In the satellite, both protomerite and deutomerite are very dark, rendering the septum difficult to trace. The nucleus is completely obliterated in the living animal. The epicyte is very thin, much less so than is usual with this genus.

EXPLANATION OF PLATE

Fig. 1.—Sporont of *Bulbocephalus wardi.*

Fig. 2.—Large free trophozoite of *B. wardi,* with epimerite.

Fig. 3.—Smaller trophozoite of *B. wardi.*

Figs. 4, 5.—Sporonts of *Bulbocephalus elongatus.*

Fig. 6.—Free trophozoite of *B. elongatus.*

Figs. 7, 8.—Sporonts of *Pyxinia bulbifera.*

Fig. 9.—Protomerite of *P. bulbifera,* to indicate thickening of epicyte and indentation left by epimerite.

Fig. 10.—Epimerite of *P. bulbifera,* not expanded.

Fig. 11.—Epimerite of *P. bulbifera,* with bulb partially expanded.

Fig. 12.—Epimerite of *P. bulbifera,* bulb fully expanded.

Figs. 13, 14.—Two associations of *Gregarina neglecta.*

Fig. 15.—Protomerite of primite of *G. neglecta,* to indicate papillate apex and thickening of epicyte.

Fig. 16.—Protomerite of satellite of *G. neglecta.*

Figs. 17, 18.—Two associations of *Gregarina polymorpha.*

Fig. 19.—Protomerite of primite of *G. polymorpha.*

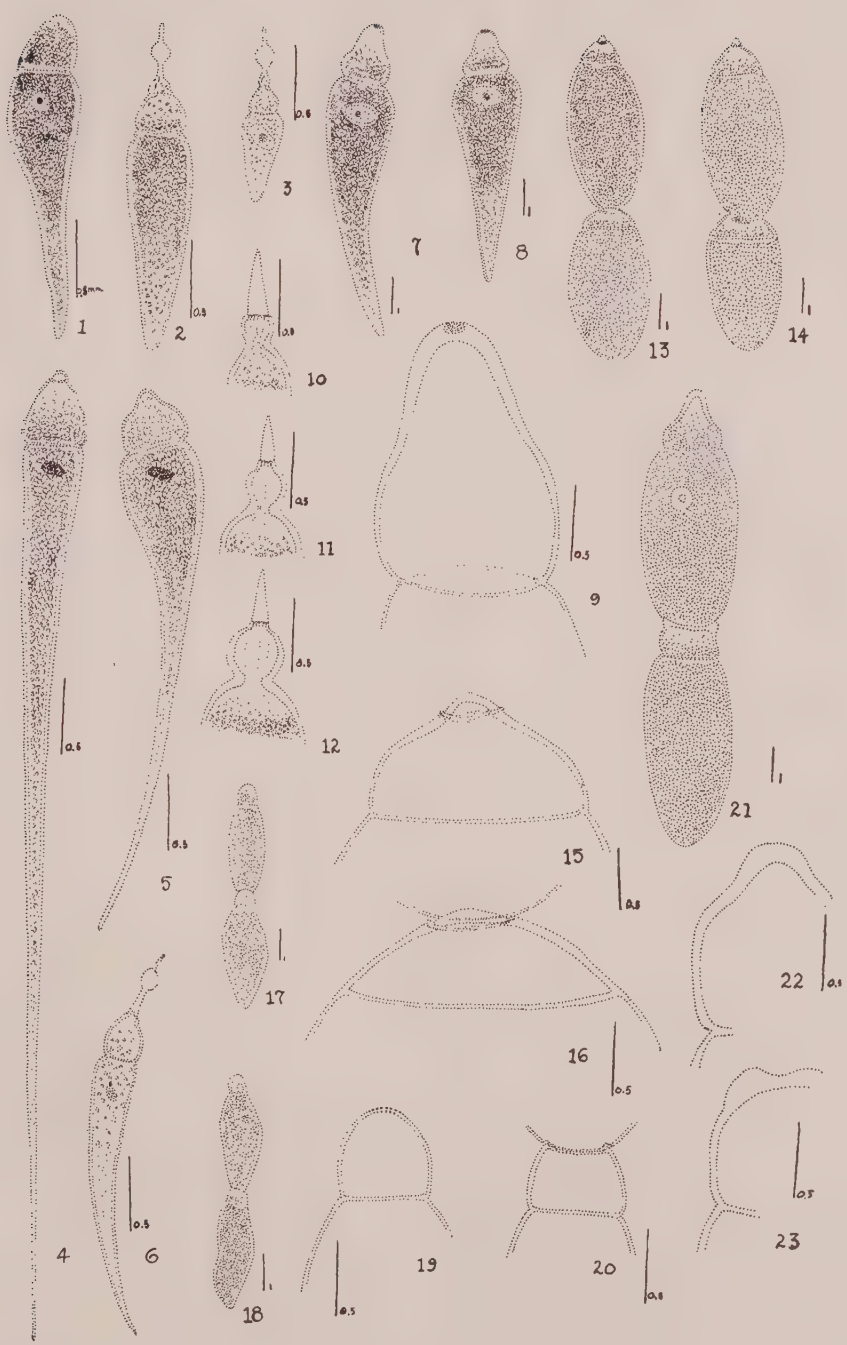
Fig. 20.—Protomerite of satellite of *G. polymorpha.*

Fig. 21.—Association of *Gregarina blattarum.*

Fig. 22.—Protomerite of primite of *G. blattarum,* to show thickening of epicyte at apex.

Fig. 23.—Protomerite of satellite of *G. blattarum.*

PLATE



Cysts were found in three stages of development, measuring in internal diameter about 300μ (see table of dimensions). No spores were seen.

Gregarines have been described from the genus Ceuthophilus in three cases: two by Ellis, and one by myself (Watson, 1916: 114-5). The present species is differentiated from the first two species in shape, from the one in shapes of the two protomerites in the association, from the other in shapes of the deutomerites. It differs from the last described species in size of sporonts and cysts and in relative thickness of the epicyte, the associations of the present species being approximately three times the length of those of the species formerly described, and the cysts twice as large in the present species (150μ and 300μ in diameter, respectively).

A table of measurements is appended, dimensions being stated in microns:

	Primite				Satellite			
	a	b	c	d	a	b	c	d
Length protomerite	70	100	70	70	50	40	50	60
Length deutomerite	430	400	390	400	350	330	370	370
Width protomerite	130	150	120	120	170	190	130	150
Width deutomerite	210	230	210	190	200	210	200	200
Total length sporont....	500	500	460	470	380	370	420	430
Ratio length prot.: total length	1:7.1	1:5	1:6.5	1:6.7	1:7.6	1:9.2	1:8.4	1:7.1
Ratio width prot.: width deut.	1:1.5	1:1.5	1:1.7	1:1.6	1:1.2	1:1.1	1:1.5	1:1.3
Total length association.	880	870	880	900				
Diameter cysts:								
Without envelope...	300	310	290					
With envelope.....	none	470	none					

GREGARINA POLYMORPHIA (Hammerschmidt) Stein

[Figures 17, 18, 19, and 20]

Host: Larva of a Tenebrionid, probably *T. molitor*
Region of infection: Intestine
Location: Oyster Bay, Long Island, August, 1916

One specimen of this Tenebrionid beetle larva from a meal sack was found to contain half a dozen associations of one of the gregarines.

The specimens are characterized by a regularly curved protomerite in the primite and a protomerite of the same shape, but slightly flattened, in the satellite.

The sporonts vary in length from 280μ to 320μ, in width from 100μ to 120μ. The ratio of length of protomerite to total length of sporont is approximately 1:7 for both primite and satellite; the ratio for width of protomerite to width of deutomerite is about 1:2.

The sporonts are small, elongate ellipsoidal with a relatively small protomerite. The protomerite is beautifully rounded in outline in the primite, representing about two-thirds of a sphere. The widest part

is just below the middle, and there is a distinct constriction at the septum. The protomerite of the primite is approximately as wide as high; while that of the satellite, which is the same shape but flattened at the apex, is wider than high. The deutomerite is widest at about its mid-region, tapering anteriorly to the septum and posteriorly, ending in a long blunt cone.

The protoplasm in the sporont is abundant, rendering it dark brown, almost black in the deutomerite and dark tan in the protomerite. No nucleus is visible in either member of the adult association. The ectosarc is especially thin, even at the tip of the protomerite and at the septum, causing rupture to be easily effected in water, an unnatural medium.

The animals are relatively active in movement, both of progression and contortion.

Neither cysts nor spores were seen.

This species has been described from Europe and Japan, but not heretofore from the United States. The first mention of the species was made in 1838 by Hammerschmidt, and it has been seen at various times since then, by Stein, Frantzius, Schneider, Léger and Duboscq, Ishii, etc.; see Watson, 1916: 172, for correlations and references. No two descriptions and drawings tally exactly, but there is not sufficient evidence to differentiate the observations into separate species. The present detailed description for a known species is given because here also are indicated variations from the orginal of Hammerschmidt, and since the specimens seen by every worker differ slightly from those of the others, it does not seem wise even to describe a new variety.

A table of a few typical measurements follows, dimensions being in microns:

	Primite				Satellite			
	a	b	c	d	a	b	c	d
Length protomerite	60	40	40	40	40	40	40	40
Length deutomerite	250	270	240	280	280	280	240	270
Width protomerite	60	50	50	40	70	60	40	60
Width deutomerite	110	110	100	120	120	110	100	120
Total length sporont....	310	310	280	320	320	320	280	310
Total length association.	630	630	560	630				
Ratio length prot.: total length	1:5.1	1:7.7	1:7	1:8	1:8	1:8	1:7	1:7.7
Ratio width prot.: width deut.	1:1.8	1:2.2	1:2	1:3	1:1.7	1:1.8	1:2.5	1:2

GREGARINA BARBARARA Watson 1915

This species was found at Oyster Bay, Long Island, in *Adalia bipunctata* in June, 1916. This, our commonest lady beetle in the East, was examined frequently throughout the year, but was found parasitized but once. Two associations and half a dozen isolated sporonts

were found. This species has heretofore been recorded from an unidentified Coccinella, not the present species.

A few measurements, in microns, are appended to supplement those already given (Watson, 1915: 31 and 1916: 185), and for sporonts somewhat smaller than those previously described.

	Primite				Satellite
	a	b	c	d	a
Length protomerite	20	30	20	20	8
Length deutomerite	80	70	70	80	52
Width protomerite	30	30	30	30	30
Width deutomerite	55	60	45	55	40
Total length sporont	100	100	90	90	60
Total length association	160				
Ratio length prot.: total length	1:5	1:3.3	1:4.5	1:5	1:7.5
Ratio width prot.; width deut	1:6	1:2	1:1.5	1:1.6	1:1.3
Diameter nucleus	....	12			

GREGARINA BLATTARUM Siebold

[Figures 21, 22, and 23]

Host: *Blatta orientalis* Linn
Regions of infection: Intestine and rectum
Location: Urbana, Illinois, June, 1915

A dozen or more biassociative gregarines were found in one specimen of the Oriental cockroach, this being the greatest number found by the writer in a single host. Many roaches were examined with negative results.

The insects are also parasitized by two species of nematodes, an infusorian, and an amoeba, the last two of which were described by Leidy in 1877 and 1881.

This gregarine is characterized by long, slender sporonts, blunt at the posterior end, by a conical, or papillated, protomerite in the primite, and a broad, flattened protomerite in the satellite.

The sporonts vary in length from 510 to 1100μ, and in width from 160μ to 400μ. The average ratio of length of protomerite to total length of sporont is 1:5 for the primite and 1:8 for the satellite. The protomerite of the primite is approximately two-thirds as wide as the deutomerite, that of the satellite approximately three-fourths to fully as wide as the deutomerite. The ratio in the primite of width of protomerite to width of deutomerite is about 1:1.7, in the satellite about 1:1.4.

A table of representative measurements is appended herewith.

The protomerite of the primite is bluntly pointed, the ectoplasm at the apex being a much thicker layer than elsewhere in the animal. The widest portion of the protomerite is about two-thirds of its length from the apex, and there is a slight constriction at the septum separating protomerite and deutomerite.

The deutomerite is elongate ellipsoidal, varying but little in width throughout the length, and broadly rounded at its posterior end. The end attached to the satellite is but little flattened.

The protomerite of the satellite is slightly flattened anteriorly, and there is but little or no constriction at the septum. The deutomerite is more or less irregularly shaped, ending in a rather blunt point.

The nucleus is small and spherical. It measures about 90μ in diameter in sporonts. The spherical karyosome is faintly visible.

The deutomerite of the sporonts is very dense and blackish, the protomerite slightly less dense and dark tan in color. The deutomerite is finely granular and homogeneous, and is slightly more dense in the satellite than in the primite. The nucleus was not visible in the satellite of any specimen seen. The protomerite contains large spherical masses less closely packed together than in the deutomerite.

This species was found first by Siebold in Germany, and by many subsequent workers, including Frantzius, Leidy, Schneider, Marshall, de Magalhaes, and Crawley, and from Germany, France, Brazil, and Pennsylvania (see Watson, 1916: 99-100).

The present specimens have the same general proportions as those already described, but reach a much greater length than that stated by Leidy, which is 500μ for a single sporont; no other writer has given dimensions. The specimens now described are undoubtedly closely related to the species he saw and described as *Gregarina blattae orientalis,* for he mentions the "slight papillary thickening of the integument" at the apex of the protomerite and indicates this feature in his three drawings. He does not state, however, whether or not the species is associative.

Because of the very considerable confusion surrounding this classical species in the past, it does not seem to the writer wise to add to it and describe the specimens now found as a variety of the type species when the only disparity is found in a papillate or non-papillate apex of the protomerite. A number of variations have already been described, but are now separated into species.

The measurements given below are in microns.

	Primite				Satellite			
	a	b	c	d	a	b	c	d
Length protomerite	120	150	160	200	60	80	100	160
Length deutomerite	390	720	790	900	460	750	600	870
Width protomerite	120	160	200	200	150	160	250	250
Width deutomerite	200	260	300	360	200	230	250	400
Total length sporont....	510	870	950	1100	520	830	800	1030
Ratio length prot.: total length	1:4.2	1:5.8	1:5.9	1:5.5	1:8.6	1:10	1:8	1:7
Ratio width prot.: width deut.	1:1.7	1:1.6	1:1.5	1:1.8	1:1.3	1:1.4	1:1	1:1.6
Diameter nucleus		90						
Total length association.	1030	1700	1750	2130				

A new place record has been established for *Leidyana erratica* (Crawley) Watson, at Douglas Lake, Michigan, by Mr. H. G. May, of the University of Illinois. This species and *Gregarina oviceps* Diesing were very commonly parasitic in the same host, which has been designated *Gryllus americanus* Blatch. Mr. May says, however, that the host may be *G. pennsylvanicus* Burm., or even an hitherto undescribed species of cricket. The cricket fits neither description perfectly.

In none of the instances recorded above was it possible to complete a life-history of the parasite because the hosts are for the most part uncommon and rarely more than one specimen of the same species was taken during the summer. It was unfortunately impossible to secure any intestines for sectioning purposes and, as cysts were rarely seen in the host parasitized, the life-histories were not carried to completion.

Type specimens of the above species have been deposited in the Ward Collection of Parasites at the University of Illinois.

SUMMARY

A new genus, Bulbocephalus, with two new species is described for the family Stylocephalidae.

New species are described for Pyxinia and Gregarina, and new data are given for Gregarines already known.

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ON A TREMATODE LARVA ENCYSTED IN A CRAB, *HELICE TRIDENS* (DE HAAN)

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In November, 1915, a considerable number of the encysted larvae of a trematode and some specimens of an infected crab were forwarded for identification by T. Urita, a friend of mine in Kagoshima, with a letter which read as follows: "The liver and the inner surface of carapace of a crab, *Helice tridens* (de Haan), are heavily infested with the egg-like cysts with thick, transparent wall containing a moving worm which seems to be a trematode larva. Some worms creep out. The crabs are found abundantly in the salt-field living in the holes they dig. Seventy to ninety per cent or more among the crabs in the vicinity of Kagoshima City are infected with the cysts."

Afterwards I obtained the crabs of Kagoshima Prefecture on several occasions from the same friend and from H. Yamakado, a student of our academy, and collected them myself on the seashore near Osaka. The crabs are found in various districts, especially in the warm parts of this country. They live in ground near the seashore, such as salt-fields, river mouths, and other coast areas exposed when the tide ebbs, and habitually build burrows in which they live. The crabs are not used as food in Japan proper, but they are said to be eaten in some parts of Korea and Formosa. There is no need to describe the morphological characters of the crab here.

On several occasions I examined two hundred and fifty specimens of the crabs and found about 90 per cent of them infected with the cysts. There is no local difference in frequency of infection between the crabs collected near Osaka and those of Kagoshima. The number of cysts harbored in a crab is variable but generally large, varying from one to several hundreds, though sometimes they are in masses by the thousand. The encysted larvae occur generally in the ovary, on the wall of the stomach, in the hypodermis lining the carapace, and other parts of the body cavity of the host. The ovary (Fig. 1*A*) and hypodermis are most heavily and frequently infested with them. In the liver I have rarely found the cysts, although T. Urita informed me of the liver infection in his letter quoted above. I found occasionally, however, infected pieces of membranous tissues twining round the lobes of the liver, a condition which perhaps would suggest liver infection.

Morphology of the Encysted Larvae.—The encysted larvae found in the crab are oval or rarely spherical in shape, varying more or less in size. Measurements of eight cysts show a range in length from 0.407 mm. to 0.601 mm., and in breadth from 0.366 mm. to 0.510 mm. The average length was 0.521 mm. and breadth, 0.418 mm.

A slight pressure by the cover-glass may alter the dimensions of the cysts; the measurements given above are obtained from those not compressed.

The wall of the cyst is a transparent chitinous membrane from 0.02 to 0.04 mm. thick through which the larva may be readily observed. An actively moving larva in the fully developed cyst is light brown in color with dark spots marking the position of the yolk glands. The worm occupies almost all the space within the cyst with the body bent in various fashion (Figs. 1 *B, C*). In the most common position in the cyst, either the anterior or posterior extremities of the body, or both, coil ventrad, and both lateral margins of the anterior region are also sometimes bent over the ventral surface. Through the wall of the cyst one may recognize the organs of the larva, i. e., oral sucker, alimentary tract including pharynx, long esophagus, intestinal ceca, excretory vesicle, and such genital organs as ovary, yolk glands and testes; but the identification of these organs is complicated by the movements of the body.

The encysted larvae in full development may emerge from the cysts at various times after the latter are removed from the crab and put in distilled water or physiological salt solution. Sometimes this takes place within only ten to thirty minutes after the cysts are removed from the host. Cysts from dead crabs are generally somewhat weakened, in consequence of which the larva may easily emerge from the cyst. Encysted larvae from crabs that have been dead a long time are also dead or so strongly affected as to be at the point of death. It is believed that the action of the putrescent fluids explains the fact that the larvae are generally found out of the cyst and dead. During my study I often found the larvae free and dead after a day or two in culture dishes.

External Feature of the Larva Liberated from the Cyst.—The larva freed from a fresh cyst is actively mobile, changing its shape and size greatly, especially in the anterior region. Figure 2 *B* serves to show the extent of change in the outline of a worm in the contracted state. On the whole, it is concave on the ventral surface and convex on the dorsal; an accurate measurement of length and breadth is very difficult when the worm is alive. The most natural form, however, is to be found in a larva that has recently died a natural death. The larva put between the slide and cover-glass also assumes a form resembling

the natural, though slightly exaggerated. The most common form then assumed is an elongated oval, the middle one-half or two thirds of the body length being of nearly uniform breadth. Both extremities taper, the posterior being more obtuse than the anterior end. In seven fixed specimens measured, the length varied from 0.533 to 0.833 mm., and the breadth from 0.283 to 0.325 mm., the average dimensions being 0.701 by 0.301 mm. Two living specimens measured, respectively, 1.0 by 0.50 mm. and 0.922 by 0.43 mm. Four specimens compressed and mounted in potassium acetate varied from 0.9 by 0.466 mm. to 1.18 by 0.56 mm., with an average of 1.083 by 0.515 mm. Five specimens compressed and mounted in Canada balsam varied from 0.75 by 0.35 mm. to 1.07 by 0.50 mm., with an average of 0.918 by 0.438 mm.

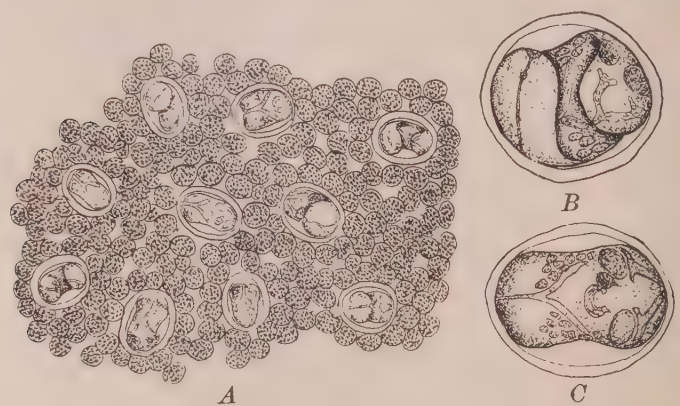


Fig. 1.—*A*, portion of the crab's ovary infested with cysts. × 23. *B*, *C*, two encysted larvae. × 60.

The entire surface of the body is armed with minute spines which are denser in the anterior region than in the posterior and inconspicuous in mounted specimens. The oral sucker (Fig. 2 *B*) is subterminal and spherical or ovoid in shape; its aperture varies according to the state of contraction of the organ. The ventral sucker (*B*) situated about one-third the body length from the caudal end is oval or spherical and smaller than the oral sucker. In the living specimen it is usually indistinct. The average dimensions of the oral sucker in compressed living specimens were 0.053 by 0.05 mm.; in specimens mounted in potassium acetate, 0.0693 by 0.0625 mm.; in specimens mounted in Canada balsam, 0.052 by 0.055 mm. The average dimensions of the ventral sucker in compressed living specimens were 0.037 by 0.024 mm.; in specimens mounted in potassium acetate, 0.035 by 0.040 mm.

Internal Structure.—The internal structure of the larva (Fig. 2) is easily observed in the living specimens compressed or in the mounted

specimens. The larva is remarkable in that almost all the essential genital organs are well developed.

The prepharynx (*P*) is distinct but short and slender, 0.03 to 0.08 mm. long and 0.01 to 0.02 mm. broad. The pharynx (*H*) is subspherical, 0.035 to 0.05 mm. long and 0.025 to 0.048 mm. broad. The esophagus (*E*) is very long and slender, 0.25 to 0.35 mm. long, with the breadth gradually increasing posteriorly to a maximum of 0.015 to 0.027 mm. The intestinal ceca (*C*) are short, running from the posterior end of the esophagus obliquely postero-lateral, so as to form a V-shape, the distal ends being far from the lateral margins of the body. The length of the ceca on both sides is nearly equal in the same individual and slightly variable in the different specimens. (0.2 to

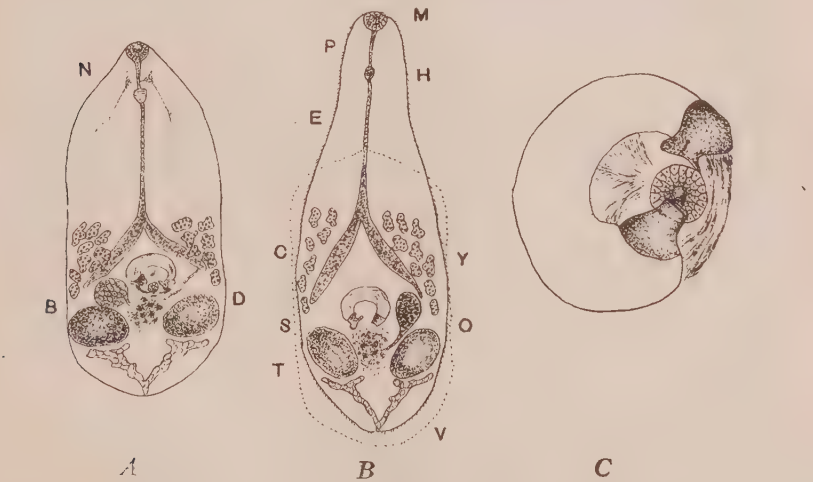


Fig. 2.—*A*, specimen mounted in potassium acetate. × 60. *B*, living larva in motion. × 60. *C*, semilunar organ and ventral sucker. × 280.

0.3 mm. long). The breadth is usually maximum at the middle part of the cecum, but it may be variable according to the condition of its contents.

The excretory vesicle (*V*) is V- or Y-shaped, and each inner end gives off two branches. The winding and branching canals arise from the anterior end of the vesicle, but their entire course cannot be made out distinctly.

The cerebral ganglion (*N*) surrounding the prepharynx and the lateral nerves from it may be observed in good specimens.

Genital Organs.—Two testes (*T*) lie side by side near the posterior end of the body, just in front of the excretory vesicle. They are oval and subequal with the long axes transverse or obliquely to the axis.

They measure 0.15 to 0.26 mm. in length and 0.09 to 0.16 mm. in breadth. The vas deferens arises from the mesial surface of each testis, but its further course cannot be traced. Just anterior to the ventral sucker there is a peculiar organ (*B*) resembling the cirrus sac. It is semilunar, lying transversely with concave side posterior; each extremity is provided with a chitinous process of form unlike the other. The chitinous process on one side (usually the right) tapers from its wide base and terminates in a smaller single tubular end, while the distal end of the process on the other side is divided into smaller unequal shaped processes (Fig. 2 *C*). The free ends of both chitinous processes face each other across the ventral sucker. The significance of this semilunar organ cannot be determined and no connection with other organs was determined. Before and behind the ventral sucker there are muscle fibers connecting with the semilunar organ.

The ovary (*O*) lies on one side (usually the right) of the median line and in the space bounded by the semilunar organ, the posterior end of the intestinal cecum, and the testis on the same side. It is oval or spherical in shape, being 0.07 to 0.12 mm. long and 0.07 to 0.1 mm. broad. The short oviduct springs from its postero-mesial aspect and runs postero-mediad to a cell-group situated in the space between both testes and the semilunar organ. This probably represents the shell gland and the uterus. Here one may find granules very similar when stained to the yolk granules. The cell-group is deeply stained by carmine.

The yolk glands (*Y*) occupy a small area on the antero-lateral aspect of each cecum. The glands on each side consist of from nine to eleven follicles on the ovarian side and from seven to nine on the other side. These follicles are irregular in form and vary slightly in size; but the maximum diameter varies between 0.055 and 0.08 mm. and the minimum between 0.027 and 0.045 mm. The yolk duct (*D*) from the glands runs postero-mediad to the cell-group mentioned above. The genital aperture could not be detected.

From the characteristics mentioned above it is difficult to determine the adult form corresponding to the encysted larvae. I endeavored to find the matured form by animal experiments. On November 26, 1915, many cysts from the crabs were fed to three guinea-pigs, and on December 14 a number of others to two young cats. These animals died or were killed after several days and were carefully examined for distomes, but in vain. On February 5, 1916, several cysts were eaten by three guinea-pigs, but they did not develop. On April 25 cysts were given to five frogs. After four or five days the frogs were killed and examined for the distomes. In the stomach, intestine, and cloaca

larvae were found free from the cysts and dead. Thus far in animal experiments I have not succeeded in obtaining the adult.

These encysted larvae evidently differ from the adult less than in most other cases, because the genital organs are so well developed. The alimentary tract, long esophagus, short intestinal ceca and the position of the yolk glands suggest a relationship to the genus Brachycoelium. Other feeding experiments with the encysted larvae are under way at present and will be described later fully with the microscopical structure of the worm.

CYTOLEICHUS PENROSEI, A NEW ARACHNOID PARASITE FOUND IN THE DISEASED LUNGS OF A PRAIRIE DOG, *CYNOMYS LUDOVICIANUS*

FRED D. WEIDMAN

On March 28, 1907, a male prairie dog, *Cynomys ludovicianus*, died in the Philadelphia Zoological Gardens with acute bronchopneumonia. The inflammatory condition was recognized at the time of autopsy, the lungs being described as diffusely red, standing out well, their apical parts emphysematous and cut surface showing minute white projecting areas. The parasites forming the basis of this communication were not recognized at this time on account of their extremely small size, being among the smallest of which the writer has found record, and much smaller than the ones which were found here (Weidman, 1915) in the lungs of a monkey and upon birds. The skin bore, especially around the head and shoulders, brownish crusts which were tightly adherent to deeper parts and which, when examined microscopically, were found to contain a fungus.

The gross diagnosis of bronchopneumonia was confirmed microscopically, shown by the presence of numerous red blood cells, frequent clumps of fibrin and moderate numbers of leukocytes in the air sacs, with which a greenish brown granular material, doubtless the excrement of the mites, was intermixed. In addition to the inflammatory disease, the sections showed a high grade of emphysema and bronchiectasis (Figs. 14, 15 and 16).

That articulated parasites were also present was recognized in the microscopical sections which shortly and routinely followed the autopsy in 1907, but it was only during a recent review of prairie dog tissues in connection with another parasite of prairie dogs [*Hepaticola hepatica* (Bancroft, 1893) Hall, 1916] that their arachnoid nature was determined. During this interval (about nine years) the tissue had been preserved in alcohol, precluding experiments on transmission of the infestation and observation of living specimens, but not interfering with staining qualities of sections. These, in favorable cases, submitted parasites showing parts of all four legs (Fig. 15) of one side, thus allowing the diagnosis of arachniasis from microscopic sections alone. They are present in large numbers, almost every section containing at least part of one and generally several, and lie for the most part within air sacs, less often in bronchi. They are surrounded by no special

grade of either acute or chronic tissue reaction such as was found in cases of Wellman and Wherry (1910).

It is impossible to state how common the infestation is in these animals because so few come to autopsy. The beasts rarely die upon the surface, doubtless seeking seclusion under ground when they become sick, there to remain until they die. As a result, only two have come to autopsy in the last eleven years in spite of the numbers always on exhibition, and of these, only the one showed pulmonary parasites.

The material for the determination of the new species was obtained by finely teasing a small portion of the lungs, yielding some fifty or more fully developed specimens and no larvae or ova. Of these, the females were more numerous than the males in the proportion of two to one. They had been fixed in formaldehyd, preserved nine years in alcohol (70 per cent.), and were examined after clearing by glycerin or Farrant's medium. From the proportion of tissue examined to the total lung substance the lungs must have contained from one to several thousand parasites.

THE FEMALE

Females selected at random measured as follows:

Pubescent Females	Ovigerous Females
0.170 x 0.087 mm.	0.200 x 0.120 mm.
0.185 x 0.085 mm.	0.193 x 0.102 mm.
0.175 x 0.087 mm.	0.185 x 0.105 mm.
0.180 x 0.090 mm.	0.190 x 0.109 mm.
0.170 x 0.100 mm.	0.195 x 0.103 mm.

A female (Fig. 1) observed laterally measured 0.190 mm. in length and 0.094 mm. dorsoventrally.

The body is broadly oval, not quite twice as long as broad, not constricted, and broadest at about the middle.

It bears a dorsal shield of subtriangular form with broadly rounded angles whose base lies at a line between Coxa II anteriorly and apex almost at anus, posteriorly. In the latter region the boundary is not sharply marked because the confines pass so gradually into the general integument, but laterally it is, the shield curving ventrally here for a short distance over the lateral parietes. Anteriorly, it shows sharp separation from the anterior part of dorsum in but few specimens, notably the immature ones. With mature specimens its anterior border appears as an anteriorly directed, more or less gentle slope, and when this is very gentle the dorsum may appear to be covered by a subrhomboidal instead of a subtriangular shield with rounded angles. It is possible that these different appearances come about in the following way. It will be observed from the measurements given above that the mature females are of plumper form than the immature (Figs. 2

and 3), doubtless brought about by the development of the reproductive organs which produce an internal pressure resulting in expansion of the parietes. Now the anterior margin of the dorsal shield is indicated not by a ridge, nor by special difference in character of integuments (the integument is of the same character over the whole body), but by a downward curved slope (Fig. 1), the steepness of which will be more and more reduced as the foot becomes elevated by the internal pressure. The progressive reduction of this steepness will, now, with maturation, obscure the anterior margin and consequently give more and more continuity between the shield posteriorly and the anterior dorsal integument.

The dorsum carries but one pair of hairs: long, and projecting from its anterior part close to its lateral margins at a level between Coxae I and II. Dorsal pits are large, inconstant, and observed in but few (four out of fifty) specimens. Of these the dorsal shield carries two rows at the level of Coxa III; a more anterior one consisting of two pairs of well outlined pits and an inconstant lateral one, and a more posterior row of but one pair, one pit on each side. The anterior portion of the dorsum shows seven, arranged in two irregular longitudinal rows (Figs. 2 and 3).

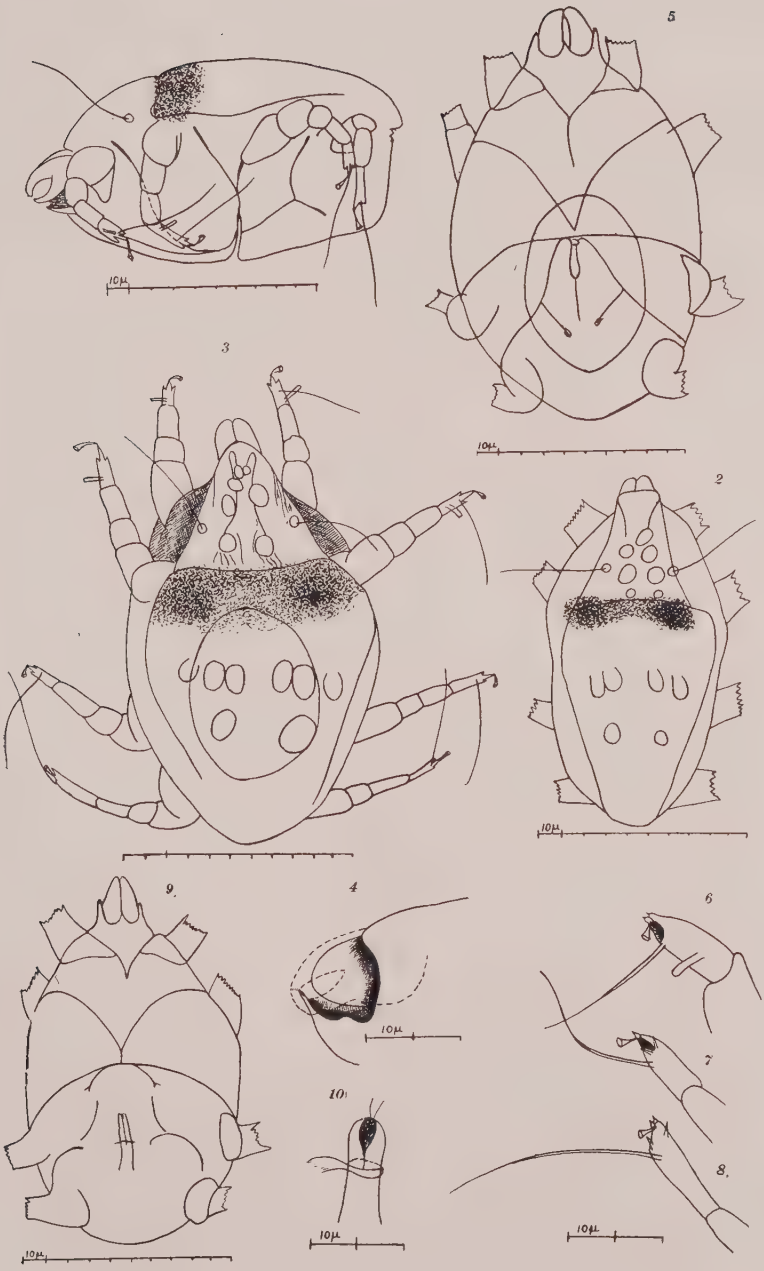
There are no eyes. The epistome is rounded anteriorly and largely covers the mouth parts as viewed dorsally. Laterally, it curves ventrally to become continuous with the hypostome, with which it forms a large tube holding the mouth parts. The hypostome shows two deep lateral scallops, with intervening median peak extending well anteriorly.

Mouth parts project but slightly beyond the body, being deeply retracted, the palpi and chelicers so closely compacted as to make their morphology indeterminate. It cannot be stated how many joints the former have, but it is most probable that they bear no hairs since some, if present, would project and be recognized in the many specimens studied. In rare cases the mandibles are recognized with untoothed chelicers (Figs. 1 and 4).

The dorsal and contiguous lateral integuments are covered by very fine, refractile, granular elevations and show no special linear markings.

The venter is divided, like that of *Sarcoptes scabiei,* into several irregular triangular areas by shallow furrows which are probably of the nature of synarthroses. They begin near the body middle, radiating laterally and curving dorsally, some nearly to the dorsal shield and all ending close to one of the coxae. (For details see Figs. 1 and 5.) The integument here, as with the dorsum, is covered by extremely fine refractile elevations of variable size, shows no linear markings, and appears to be of a soft leathery rather than chitinous nature.

PLATE 1



EXPLANATION OF PLATE 1

Fig. 1.—Lateral view of female.

Fig. 2.—Dorsum of pubescent female.

Fig. 3.—Dorsum of ovigerous female. Coxae of Legs I hidden.

Fig. 4.—The rostrum, seen ventro-laterally. Dotted lines indicate the chelate mandible seen at a lower level through overlying parts. Mandible folded in joints toward mid-line.

Fig. 5.—Venter of ovigerous female.

Fig. 6.—Tarsus of Legs I and II. The seta is foreshortened.

Fig. 7.—Tarsus of Leg III.

Fig. 8.—Tarsus of Leg IV.

Fig. 9.—Venter of male.

Fig. 10.—Male external parts.

EXPLANATION OF PLATE 2

Fig. 11.—Pubescent female. From teasings.

Fig. 12.—Ovigerous female. Also from teasings. The rounded, lighter and darker bodies are red blood cells, the darker material lung fragments.

Fig. 13.—Male. From teasings.

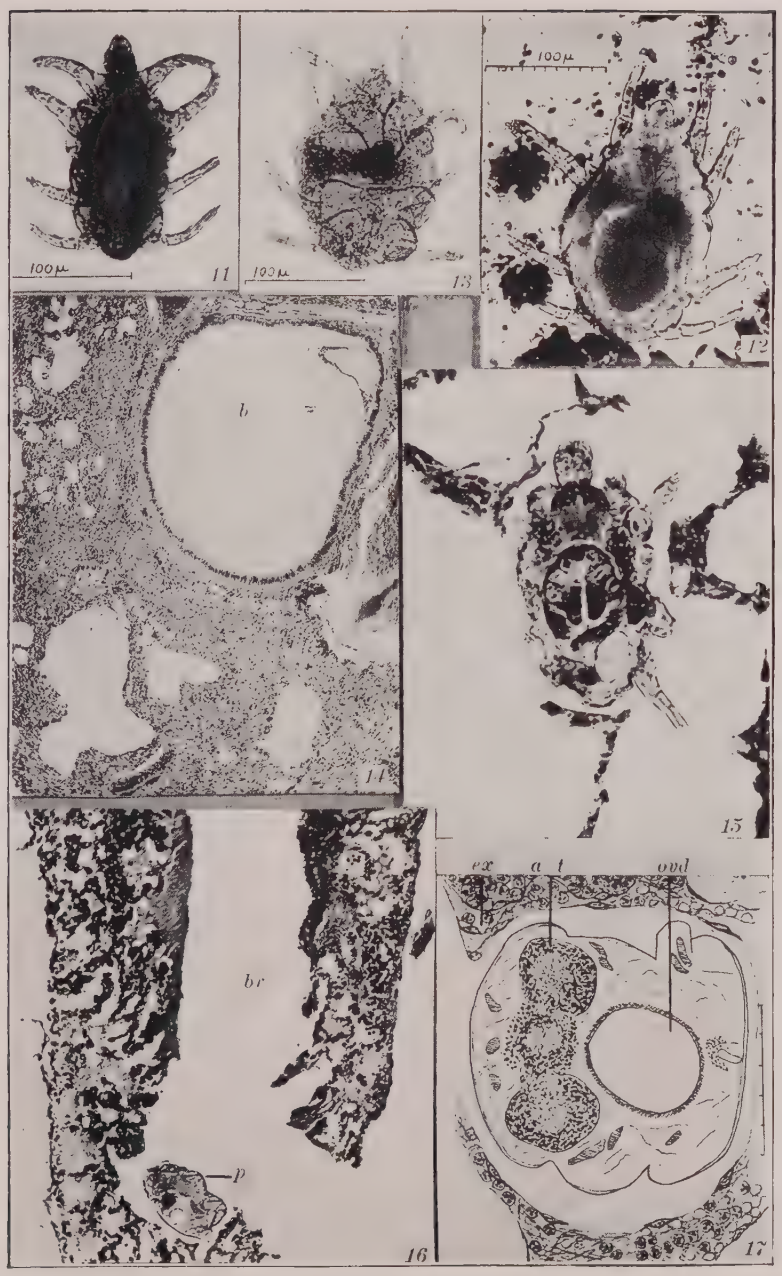
Fig. 14.—Very low-power view of microscopic section of lung showing dilatation of bronchus at *b* (bronchiectasis), irregular distention of some air sacs, and broncho-pneumonic exudate in others.

Fig. 15.—Very high-power view of microscopical section of lung showing longitudinal section of ovigerous female. Note distention of air sacs—emphysema.

Fig. 16.—Low-power view of microscopic section of lung showing parasite (*p*) in lumen of bronchus (*br*).

Fig. 17.—Transverse section through parasite in lung. *at*, alimentary tract; *ovd*, oviduct; *ex*, inflammatory exudate in lung. Each subdivision of scale equals 10 microns.

PLATE 2



The first pair of legs lies close to but not fused with the capitulum. There is a broad interval between it and the second pair, and a broader one between it and the last two pairs. The latter are separated from each other by about the same distance as the first two. As fixed, most of the specimens show the first pair directed anteriorly and the other three strongly flexed upon the coxæ and directed laterally and anteriorly, as shown in Figure 2. With many, however, the legs are flexed toward the middle of venter (Fig. 1), in which case the latter generally pouts, hill-like, strongly ventrally. The first pair is the shortest, measuring 0.07 mm.;[1] the other three but little longer, measuring 0.08 mm., and none of the four is as long as the body width. Each is composed of five segments, distinctly more slender in the last two legs than the first two, particularly the tarsus of Leg IV, which is at least four times as long as broad.

Only the tarsus bears special cuticular appendages. In the case of *all* four legs it bears the following: First, a long hair passing from a point well short of its distal extremity; second, three or four[2] minute, short, terminal spurs, the median one or two mucronate and a little in advance of the others; third, a small, delicate caroncle extending from the base of the median terminal spur. With Legs I and II only, a short, stout, rod-like seta (sense-hair) extends in addition from a point near its base (Figs. 3 and 6). Legs III and IV do not bear this structure.

The anus is terminal, at junction of dorsum and venter, and surrounded by no special appendages. Stigmal plates in spite of careful search of the abundant material, could not be found.

The vulvar orifice is ventral, median, fissural, longitudinal, lies at level of Coxa III, and ends anteriorly at the arthrosis which passes transversely at about the body middle. No ova were observed free. A specimen in the body of a female measured 80 by 65μ.

The only internal organ recognizable through the cuticle is part of the alimentary canal, the approximate position of which is indicated by thickly placed, black or brownish-black, fine to coarse granules probably consisting of altered blood pigmentary material (hemoglobin?) which has been ingested as food. It appears over a variable area extending through a greater part of the body width under the anterior

1. All measurements from an ovigerous female 0.200 mm. long unless otherwise noted.

2. The borders of the tarsus become membranaceous at the extremity and appear to be capable, either naturally or artificially, of being folded centrally. This may superimpose the central mucronate spurs so that they appear one, but in numerous cases this possibility can surely be ruled out. We are working here under very high magnification (1/12 in. oil immersion lens) where it is often difficult to translate optical appearances into positive statements.

third of the dorsal shield, and at times for a short distance beyond it anteriorly, or farther posteriorly, on each side. Transverse sections of the parasite in lung show two lateral longitudinal tubes hugging the dorsum closely which are evidently intestine, and a single larger ventral one which is probable the oviduct (Fig. 17).

THE MALE

The male differs from the female only in its slightly smaller size, minor differences in configuration of the posterior ventral synarthrosis, and its special genital organs. The measurements of several selected at random follow:

0.175 x 0.102 mm. (Fig. 8)
0.162 x 0.109 mm.
0.170 x 0.100 mm.
0.160 x 0.105 mm.
0.155 x 0.108 mm.

The genital orifice lies ventrally, median, at the level of Coxa III, is transverse, small as compared to its female counterpart, and bordered by a narrow chitinous rim. In most cases the copulatory apparatus projects from it in an anterior direction in the form of a short heavy cylindrical piece with rounded ends. The parts are so homogeneous that finer details cannot be asserted with certainty, but it appears as though the piece were a tube whose wall is split longitudinally through its whole length anteriorly, and whose lumen contains two extremely delicate, curved, sharp-pointed spicules (Figs. 9 and 10). For differences in configuration of posterior ventral synarthrosis compare Figures 5 and 8, a female and male.

PATHOGENICITY

Since knowledge of the clinical course of the disease is lacking, postmortem findings furnish of course the only basis for judging the part played by the parasite in producing death. By studying these, it was found that all of the lesions above described were acute ones, best seen in the microscopic sections, where among other changes emphysema and bronchiectasis were described, both of which are commonly produced by severe coughing.

Now, these two changes may be caused by either acute or chronic coughs. In those cases where the cough is chronic, lasting, say, several months, it is found that fibrous overgrowths occur in addition, particularly in the walls of bronchi, and that infiltrates of lymphocytes are also sometimes associated. But none of these are seen in this case. Bronchial walls are uniformly thin, and free of cells other than those which can be explained by the nearby acute inflammation. There

exists here the *acute* forms of bronchiectasis (or bronchiolectasis) and emphysema (so-called).

It has been already noted that no ova or larva were found in the abundant material studied. It may be added that there is no important difference in size between the mature specimens. These observations, together with the lack of *chronic* pulmonary tissue changes, lead to the belief that the mites were present but a short time, certainly not long enough to reproduce, and so probably not longer than a few weeks, as *Sarcoptes scabiei* matures from the larva in about three weeks. Under these circumstances it is the reasonable thing to believe that it is the parasites which have excited the acute bronchopneumonia and so induced death.

The two cases of Wellman and Wherry concerned parasites of squirrels which were well encapsulated in tubercular nodes, i. e., had been present for some time. It is possible that the squirrels, too, had suffered from an acute attack of bronchopneumonia at the time of infestation, and if this be true for them, it should also be thought that the disease produced by *C. penrosei* may also be recovered from at times and the mites encapsulated.

The original source and mode of entry are only speculative, as discussed in the case of the monkey infestation which has been referred to earlier (1915) as occurring in these gardens.

ZOOLOGICAL POSITION

Following Banks' (1905) key, this is indicated as follows: Class, Arachnoidea; order, Acarina; superfamily, Sarcoptoidea; family, Cytoleichidae. He describes the family as consisting of two species, *Cytoleichus* (formerly *Cytodites*) *nudus,* and *Laminosioptes cysticola,* and gives family diagnosis as "In skin and cellular tissue of birds. Vulva longitudinal." He does not include in this family the original type species, *C. sarcoptoides,* Megnin, 1879, perhaps on account of the scope of his paper, or perhaps because the species has been later placed in another genus. Nor does he give the generic diagnosis of Cytoleichus, which I assume to be still extant, as reproduced as follows from Megnin (1879):

"Body large, orbicular, convex above, plane below, continued anteriorly by a mobile, inclined, conical, tubular rostrum covered above at its base only by an epistome provided with no appendages like joints, etc. Legs conical, robust, arranged in two groups, a cephalothoracic and an abdominal, the first only being marginal, the epimerae of the first pair alone fused to form a sternal plate, the others free; tarsi without terminal hooks, only a ventral simple ambulacrum with cylin-

drical pedicle; the tarsus of the second pair shows at all ages in both sexes a blunt cirrus directed above and outward. Ovo-viviparous acarians. Type species, *C. sarcoptoides.* Habitat, air sacs of birds (pheasants)."

Comparison of the above diagnosis with *C. penrosei* shows several important differences, in spite of which the writer has placed this parasite in the genus Cytoleichus, mainly because it resembles the mites placed there by Wellman and Wherry (1910) as *C. banksi.* They were found in large numbers in the lungs of two California ground squirrels (*Otospermophilus beecheyi*), each within a tubercle, and occurred both on and within the lung substance. Their description is a brief one, and so far as it goes agrees fairly with this prairie dog species, but their illustration while a simple one, is of value here in that it shows (1) the joints of the last pair of legs distinctly heavier than in the prairie dog species, and (2) the intestinal markings far posterior. The type specimen is not available for original reference since it is recorded as in the collection of Creighton Wellman, who cannot be located in spite of some correspondence. From the data at hand, *C. banksi* would seem to differ from *C. penrosei* mainly in that (1) the joints of the posterior legs are thicker; (2) it bears no short sense-hairs upon the first two pairs of legs, or (3) longer ones upon the dorsum, and (4) no dorsal shield is mentioned. It is not possible that the two prominent dorsal hairs could have been overlooked by Wellman and Wherry (1910) had they been present in the squirrel species, nor scarcely the sense-hairs on Tarsi I and II; but it is quite possible that the dorsal shield might not be considered an entity by some observers. These differences determine a new species, *C. penrosei.*[3]

The writer feels that *C. banksi* and *penrosei* collectively show wide enough differences from the type to warrant the construction of a new genus to include them. Thus, *C. sarcoptoides,* the type, lives in birds, the other two in rodents; *C. sarcoptoides* measures nearly three times as large (0.570 mm. by 0.440 mm.), and does not bear the long tarsal seta common to *C. banksi* and *penrosei.* He prefers, rather, not to multiply genera, but to leave this to some systematist who will study a larger group of species than the occasional medical writer.

C. penrosei nov. spec. Specific diagnosis: Grossly invisible, broadly oval, with dorsal shield and bearing one pair of long hairs anteriorly. Legs nearly equal in length, none longer than body width, each with five joints, the tarsus of each with long hair near and delicate caroncle at tip. Tarsi of Legs I and II with short stout sense-hair near base

3. Dedicated to Dr. Charles B. Penrose, the president of the Philadelphia Zoological Society.

in addition. Vulva median, longitudinal, fissural, between Coxa III. Male genital orifice in similar position, but transverse. Females average 0.193 by 0.108 mm., males, 0.164 by 0.105 mm.

Habitat, lungs of prairie dog, *Cynomys ludovicianus*.

Type specimen in Philadelphia Zoological Gardens. Autopsy No. 1044.

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BOOK REVIEW

MEDICAL AND VETERINARY ENTOMOLOGY. William B. Herms. A textbook for use in schools and colleges as well as a handbook for the use of physicians, veterinarians and public health officials. New York: The Macmillan Company, 1915. xii + 393 pages. 228 figures. $4.00.

There have been a number of textbooks published for the use of students and others dealing with arthropods and the transmission of disease during the past five or six years. The present volume differs from the most of these in that it introduces the subject with a concise description of parasitism and the morphology of the parts that have to do with transmission. This includes a statement as to the classes of parasites, the effect and origin of parasitism, and a tabulation with figures of the systematic position of animal parasites. There is a brief discussion of the internal anatomy, classification and metamorphosis of insects, with good illustrations of the various types. It is unfortunate that the author has introduced new names for the three types, as there are too many already. In practically all the insects that have to do with the transmission of disease, the mouth-parts are fitted for piercing or sucking. The derivation of this type from the simpler biting type is shown in detail, not only for those groups that are known to suck blood, but for all others. This is supplemented by a list of the orders arranged according to their type of mouth, together with a statement as to their type of metamorphosis. Such a treatment is to be commended, for the sucking type of mouth is distinctive in form and structure in each group where it occurs. The following sixteen chapters discuss fully: how insects cause and carry disease, direct and indirect infection, external and internal parasites; the life-history, habits and relation to disease of common household insects; the biting and sucking lice infesting domestic animals and man; the life-history, habits and relation to disease of the bedbug and cone-nose; the transmission of malaria, yellow fever and other diseases by mosquitoes, their habits, life-history and control; buffalo-gnats and their relation to pellagra; the house-fly and its relation to the transmission of intestinal diseases, together with measures for its control; the blood-sucking muscids and their relation to sleeping sickness and poliomyelitis; myiasis and the bot-flies; fleas and the transmission of bubonic plague; ticks and tick-borne diseases; mites as skin parasites; and the venom of bees, wasps, spiders and scorpions. There are also included analytical tables for the identification of adult mosquitoes, the families of the dipterous larvae producing myiasis, and the families and some of the genera of fleas.

The book can be recommended for its figures, most of them new, and for its carefully prepared, well-balanced subject-matter.

NOTE

Special courses in parasitology with emphasis upon field and experimental work are announced by Dr. George R. LaRue in the program of the Michigan University Biological Station at Douglas Lake for the summer of 1917.